

# **TSE 5-Hole Box Systems**

For small laboratory animals

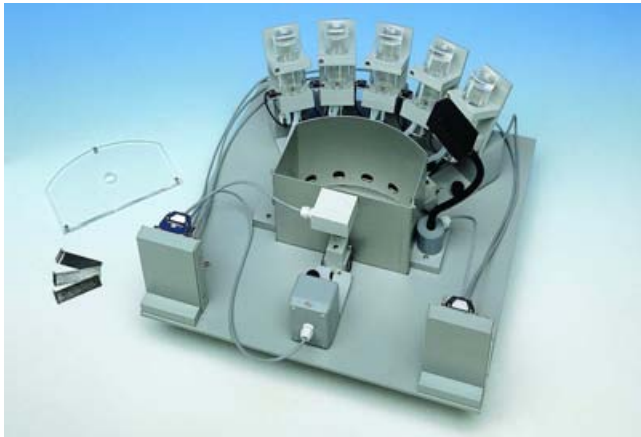
*– Specifications subject to change without notice –*





## TSE 5-Hole Box

### General information



This multi-function test box is our latest addition to our line of operant conditioning systems. It is an **attention test** based on visual discrimination (*visual attentional function test*).

The task is described in detail in "Methods for assessing attention and stimulus control in the rat", T.W.Robbins et al., Behavioral Neuroscience: A Practical Approach, Vol.1 (A. Sahgal Ed.), IRL, Oxford, 1993. Please also refer to: Humby T, Laird FM, Davies W, Wilkinson LS "Visuospatial attentional functioning in mice: interactions between cholinergic manipulations and genotype", Eur J Neuroscience 1999, Vol.11, 2813-2823.

Basically the 5-hole-box is an **operant chamber without levers**. Five circular apertures are mounted into a curved wall. Each hole is equipped with a **signal lamp**. Hole visits are monitored by an infra-red detector mounted horizontally across the opening. These are the **stimulus holes**.

During the trial visual short-lasting **stimuli** are given in pseudo-randomized order in selected stimulus holes.

If the animal nose-pokes into the correct hole, i.e. interrupts the light barrier at the entrance of the aperture, when the light is on or during a subsequent hold interval, then **reinforcement** occurs (**5-choice serial reaction time paradigm**).

The position where the reward is provided varies depending on the box configuration chosen:

- **The reward can be given in the stimulus holes.** An additional nose-poke module mounted to the front wall allows to train the animal to return to the front of the box after the reward has been given in order to ensure an optimum starting position for the next stimulus cycle. Only if the animal nose-pokes into this hole the next stimulus cycle is started.
- **Alternatively the animal has to return to the front panel in order to receive the reward** – reinforcement is given there at a single location. After the reward has been taken by the animal the next stimulus cycle is initiated.

### System components

The complete system consists of the following components:

- up to 8 test boxes for rat or mouse
- housings with ventilator and light
- a control unit
- an IBM-compatible computer with
- special interface and
- the OBS software for Windows.

### Box configurations

#### 1-Dispenser-Box



This box has **1 dispenser** to deliver reinforcement at the front wall.

- Available in rat and mouse size.
- 1-Pellet-Feeder or 1-Liquid Dispenser configuration (*please specify desired type of liquid-dispenser*).
- Both types of reinforcement devices can be easily interchanged.

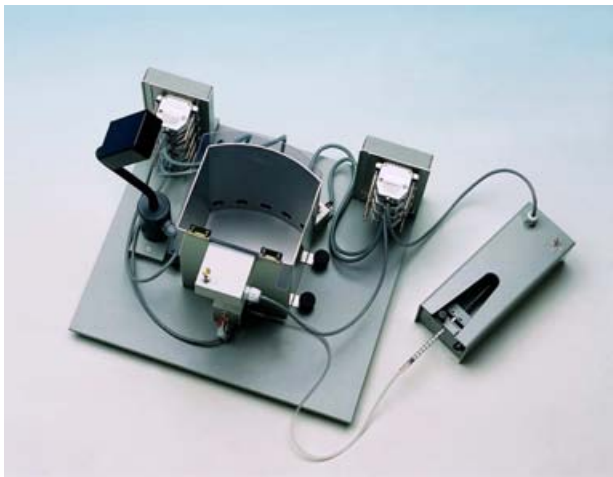
The reward-hole ("module 6") is monitored by an infra-red sensor. A nose-poke into the hole will activate the dispenser to release the reward.

A cue light can be used to signal periods where reward is available.

The hole can be equipped with a flap if desired.

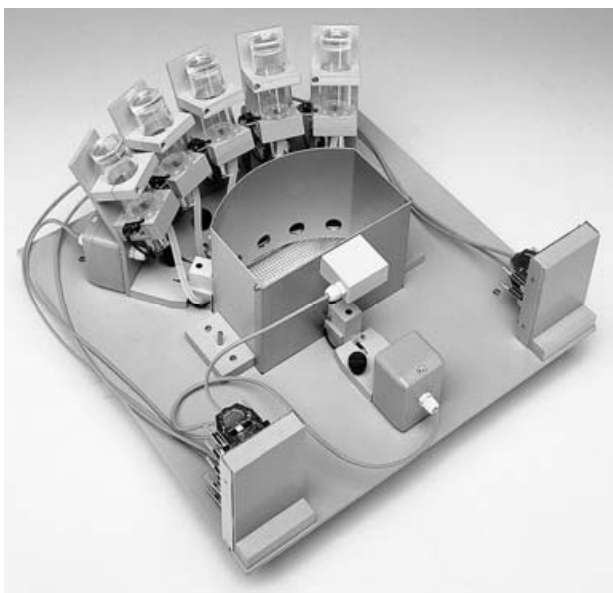


Module 6 – configured for tubing connection to the ultra-precise syringe-based liquid-dispenser



1-Dispenser-Box for mouse  
(shown with the ultra-precise liquid dispenser)

## 5-Dispenser-Box

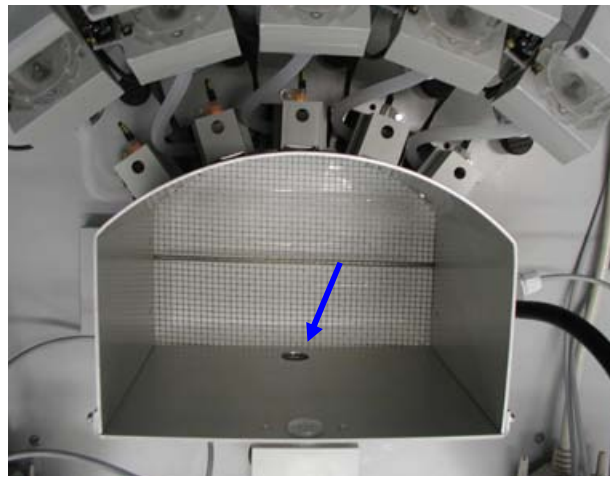


5-Dispenser-Box for mouse  
(shown with pellet feeders)

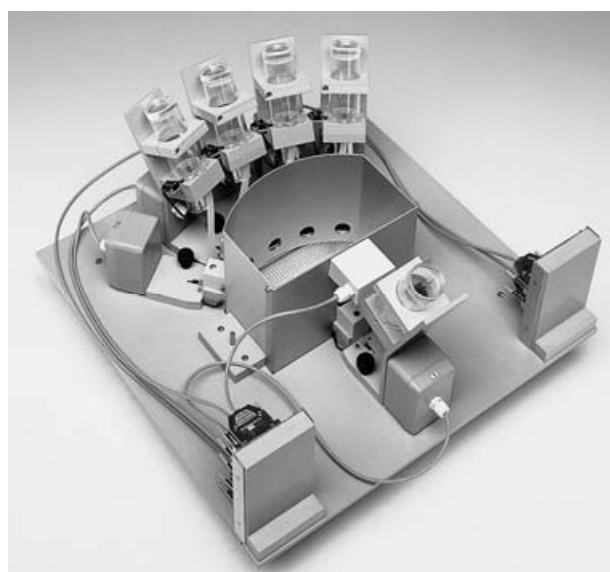
This box is equipped with **5 dispensers** located at the back wall of the box. Reinforcement is given **in the stimulus holes** (1 dispenser per stimulus hole).

- Available in rat and mouse size.
- 5-Pellet-Feeder or 5-Liquid Dispenser configuration (*please specify desired type of liquid-dispenser*).

Module 6 – the nose-poke hole available at the front wall - corresponds to the module used in the 1-Dispenser-Box. It has an infra-red sensor, a cue light and can be equipped with a flap.



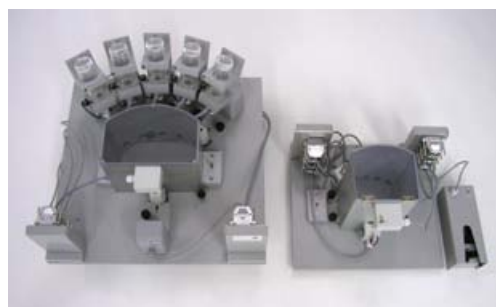
View from above: nose-poke hole 6 is mounted into the front wall



5-Dispenser-Box for mouse used as 1-Dispenser-Box  
(shown with pellet feeders)

For maximum flexibility the 5-Dispenser-Box can be converted into a “1-Dispenser-configuration”. This is done by removing one of the rear wall dispensers and mounting it to the front wall. In this configuration the box corresponds to a standard “1-Dispenser-Box” – the remaining 4 dispensers are not used in the trial procedure then.

The 5-Feeder-Box can be retrofitted with a single liquid dispenser that is mounted to the front panel. It is then used as a **1-Liquid-Dispenser-Box**. A subsequent retrofitting with 5 liquid dispensers at the rear wall is not possible!



Size comparison

**Left:** 5-Dispenser-Mouse Box (Feeder)

**Right:** 1-Dispenser-Mouse Box (Liquid Dispenser)

## Reinforcement

Food or liquid reward can be given depending on the needs of the user and the animal under study.

### 1. Food reward

Food reinforcement is given via **automatic pellet dispensers**. Our newly constructed pellet feeders are suitable for use with all standard dust-free pellets (pellet size: 20mg for mice or 45mg for rats). The durable construction ensures reliable performance for many years.



### 2. Liquid reward

Reinforcement can also be given with **liquid dispensers** in order to provide water, milk or sucrose solution instead of food pellets. Different types of dispensers are available depending on the quantities to be delivered:

- The **drop-type liquid dispenser “Advanced”** allows to deliver liquid with a minimum volume of app. **50 µl**. It is mainly suited for rats and for any non-viscous liquid such as water or aqueous solutions.
- Our new syringe-based **ultra-precise liquid dispensers** have been specifically designed for setups where very small and accurate amounts (down to 4µl) of liquids are to be delivered.



**Syringe pumps** can also be modified to be used as liquid dispensers. The volume administered depends on the [www.TSE-Systems.com](http://www.TSE-Systems.com)

diameter of the syringe used and the flow-rate selected. Please contact us for more information.

## Stimulus holes

The 5 stimulus holes are monitored by an infra-red detector mounted horizontally across the opening. The light is invisible to the animal.

Stimulus Holes “Mouse”	
Diameter	20 mm
LED color	white
LED diameter	5 mm



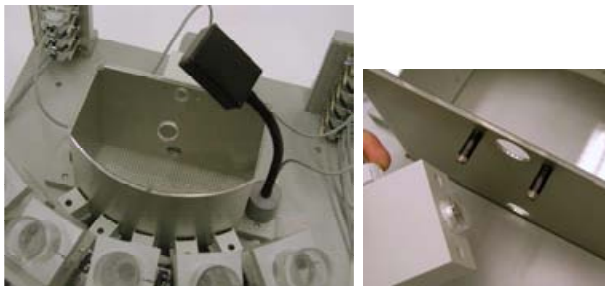
The stimulus holes can be occluded separately by metal inserts.



The stimulus hole units can be equipped with external **indicator LEDs**. If switched into the active status they are lighted up simultaneously with the stimulus lamp. This allows the observer to easily recognize the status of each stimulus lamp (on or off). This is especially useful when a camera is used to tape the experiments.



## House light



A house light is standard in all boxes. The software-controlled lamp is mounted to the front wall above the front nose-poke hole. This lamp can also be used to signal a timeout period.

House Light	
Color	white
Diameter	21 mm
Output	2 watts

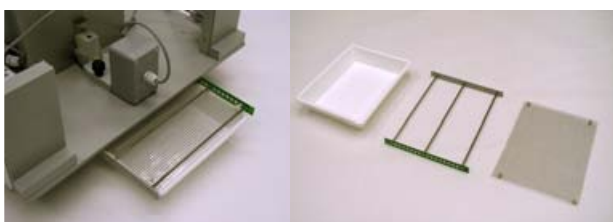
## Audio elements

In order to increase attentional load distracting sound or noise can be presented through a **loudspeaker module**. The loudspeaker is mounted to a flexible arm that can be swivelled into any position above or beside the box. The auditory signal is switched on and off via software.

Audio Elements	
	Noise: White noise with adjustable amplitude
	Sound: Sound (sine) at a fixed standard frequency of 10 kHz with adjustable amplitude

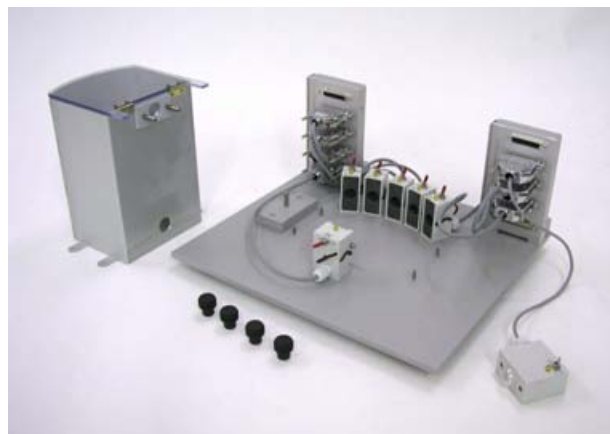
## Box floor

The standard box **floor** is made from stainless steel wire mesh. It is combined with a waste-pan made from acid-resistant material.



A metal bar grid is available on request.

Alternatively the box can be manufactured in one piece (aluminium) without removable floor. For cleaning purposes the whole box is removed from the base plate.



## Housings



The box can be operated in a sound attenuating **housing** featuring

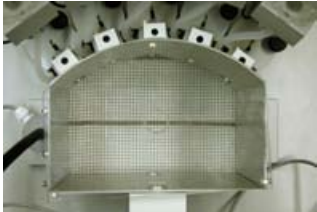
- a **fan** which provides a constant low background noise,
- a **light**, switched on and off manually,
- a sliding **floor plate** for easy removal of the box,
- a removable front **door** with integrated **inspection window**.

### Example Housing "Mouse" 5-Dispenser-Box

Dimensions	600 x 600 x 600 mm
Weight with box	Approx. 27 kg

## Options

If desired a **camera** can be mounted into the housing roof in order to observe the behavior of the animal throughout training and experiment. The box **lid** that is made of clear acrylic allows free view from above. If you want to retrofit the housing with a camera, please contact us. If the experiment is run in total darkness an infra-red camera model can be used - infra-red LEDs mounted into the roof provide appropriate illumination then.



## Control unit & PC

The control unit provides the connection between the boxes and the computer. A maximum of 4 boxes can be connected to and independently controlled by one control unit – several units can be combined with each other.



It contains the electronics for the infra-red sensors and for controlling the stimulus and reinforcer components. A built-in audio/noise generator (*optional*) controls the auditory signals (sine sound 10kHz frequency or white noise).



The front panel features 2 control knobs that are used to adjust the **amplitude** of the sound and the white noise. The scale is relative and adjusts the amplitude from zero to maximum (max. = 100 dB).

The control unit is connected to an **interface** that is built into an IBM-compatible **computer**. Requirements:

- IBM compatible computer
- An Intel Pentium CPU ( $\geq$  PIII 600MHz) is recommended
- Motherboard with 1 free ISA or PCI slot
- Operating system: Windows98SE/NT/2000

We can supply a complete system including the computer completely installed and ready for use!

## The software

The TSE Operant Behavior software controls the complete system, records the movement of the test animals and stores the measuring data for subsequent analysis.

Box control is performed using 2 different approaches:

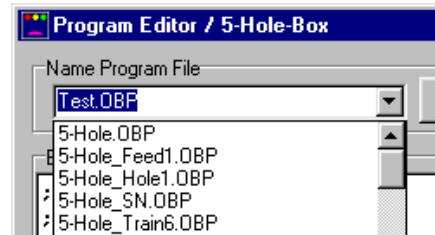
### ■ Standard paradigm

In the easy-to-use standard procedures the user can choose between a variety of pre-configured options. The software generates a table of results that discriminates incorrect, premature and correct nose-pokes and outputs average latencies for both correct and incorrect responses. A frequency distribution lists number of visits in each hole. Only the stimulus lamps are used here in order to assess sustained attention.

### ■ Free programming option

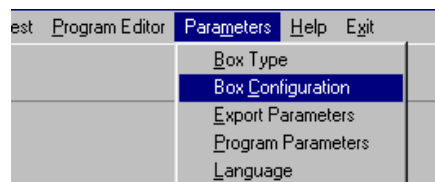
If the standard test is not sufficient then the free programming option can be used. It allows operator-defined programs to be generated. In contrast to the standard test sound or bursts of white noise can be used as distracter to additionally assess selective attention.

The OBS language is a very powerful tool for creating universal trial control programs. Programming is carried out in a language which is similar to **BASIC**. The number of commands which are available has deliberately been kept small in order to make it easier to learn.

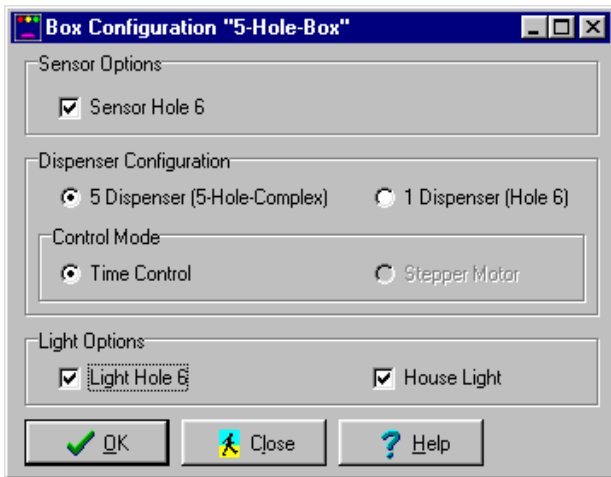


A variety of tutorial programs are provided that can be carried out directly. If you are interested in detailed information about the free programming option, please contact us. The following description refers to the standard paradigms only.

### Setting the box configuration



Before performing system tests or running an experiment the configuration of the box has to be defined in the **Box Configuration** window. The configuration affects the test procedure that can be run with the box.

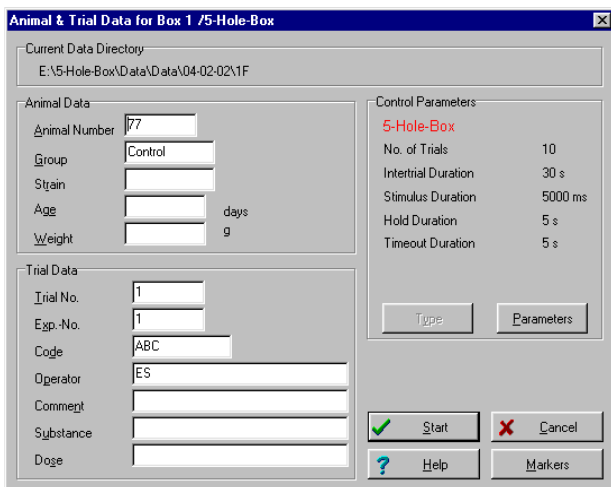


## Running an experiment

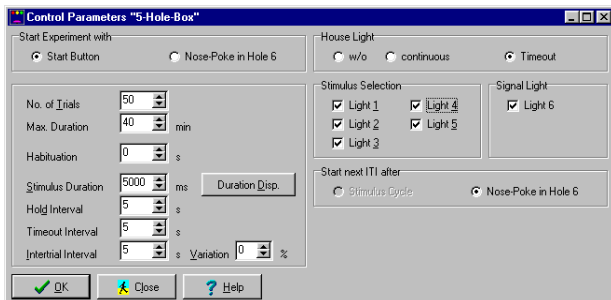
In principle an experiment proceeds according to the following scheme:

- preparation,
- execution,
- end of experiment.


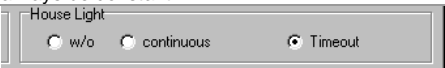
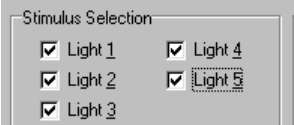
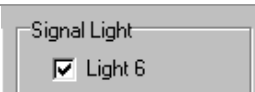
In the trial preparation phase all control and descriptive parameters are entered by the user.



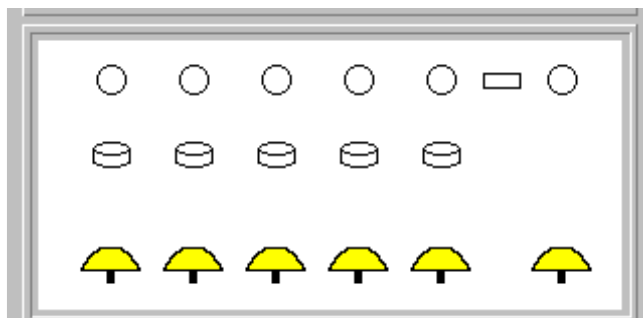
**Control parameters** influence the course of the experiment. The parameters available here depend on the box configuration chosen.



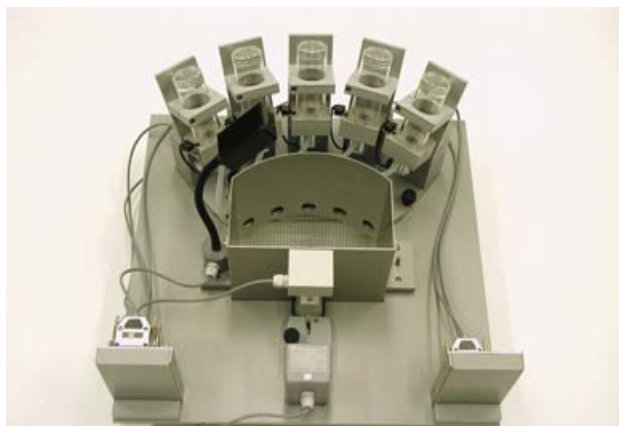
The following table describes the control parameters in detail:

<b>Start Experiment with</b>	The first ITI is either started in the moment when the user presses a START button OR when the animal <b>nose-pokes</b> into module 6
<b>No. of Trials</b>	An experiment consists of <b>N</b> trials, which are separated from each other by so-called intertrial intervals (ITI).
<b>Max. Duration</b>	This is the maximum trial duration. If this time has elapsed, the trial is terminated.
<b>Habituation</b>	In order to let the animal adjust to the box a habituation period can be defined.
<b>Stimulus Duration</b>	In each trial <b>one</b> stimulus lamp will be activated: it lights up. The duration is determined here (in milliseconds; steps of 100ms are allowed). The aim is to train the animal step-by-step to react to very short signals.
<b>Duration Disp.</b>	 <p>The duration for which the dispenser is activated is entered here. The entry effects the number of pellets or the amount of fluid delivered.</p>
<b>Hold Interval</b>	<p>The hold interval is timed from the offset of the stimulus. If the animal shows a correct response during the hold interval a reward is delivered.</p> <p><i>Example:</i> A hold interval of 20 sec means that the reward will be presented for 20 sec after the stimulus presentation has elapsed.</p>
<b>Timeout Interval</b>	If the animal shows an incorrect reaction the stimulus cycle can be interrupted for a user-defined length of time. Usually the house light is switched on during timeout.
<b>Intertrial Interval</b>	An experiment is always started with an intertrial period (if no habituation time has been defined). The length of the interval is preset here. A minimum duration of 1 second is necessary.
<b>Variation</b>	A range can be given in which the ITI length can vary (length 10 seconds and variation 50% means that the length can be anything between 6 and 14 seconds). The entry of <b>zero</b> means that the ITI will always be constant.
<b>House Light</b>	 <p>The house light can be switched OFF or ON throughout the whole experiment <b>or</b> can only be activated during the Timeout period.</p>
<b>Stimulus Selection</b>	 <p>The stimuli which are to be used during a trial are determined. At least <b>one</b> stimulus must be selected here. The stimuli are presented in a pseudo-randomized manner in the selected holes, i.e. with an experiment the stimulus will be presented with <b>equal frequency</b> in each hole. The presentation <i>sequence</i> of the stimuli is random. In this way correction trials can be carried out on animals which have developed a position bias by e.g. presenting stimuli only in the 2 right-hand or 2 left-hand holes.</p>
<b>Signal Light</b>	 <p>The wait phase can be signalled by the light mounted into module 6.</p>
<b>Start next ITI after</b>	ITI 2...N is started when the animal <b>nose-pokes</b> into module 6. For training purposes module 6 can be deactivated – then the next ITI is started when the stimulus cycle is finished.

After the test preparation has finished, the animal is placed in the cage and data acquisition is started in this specific box.  
 During the trial a schematic diagram of the boxes connected is shown – the **trial monitor**.



Trial Monitor Standard Paradigms  
 Shown is the display for a 5-Dispenser-Box

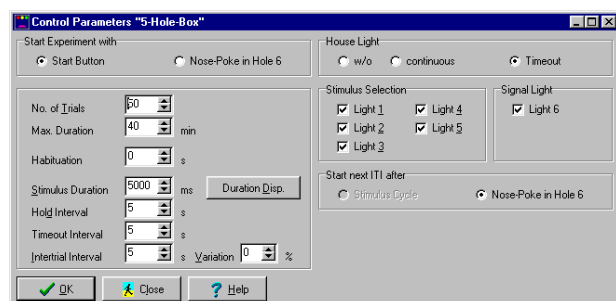


5-Dispenser-Box

## Description of the 5-hole box paradigm

In the following example the trial procedure in a **5-Dispenser-Box with Feeders** is described as an example.

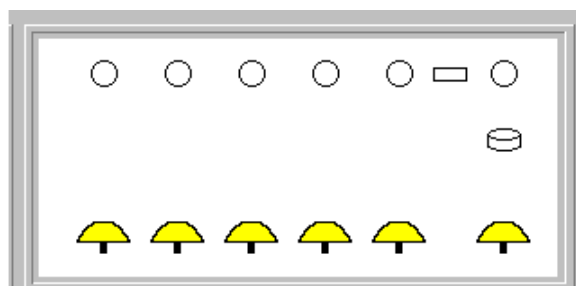
<b>Start options</b>	Start Button / Nose-Poke in Hole 6
<b>ITI options</b>	Nose-Poke in Hole 6



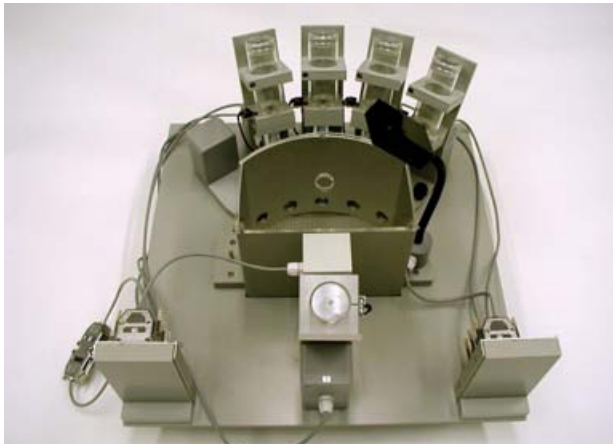
- After pressing the **START** button the **habituation** time starts first – if defined.
- Then the **intertrial interval** of trial 1 starts immediately (**Start option START button**) OR the system is in a **WAIT phase** and waits for the animal to interrupt

sensor 6 in order to start the first ITI (**Start option Nose-Poke in Hole 6**).

- When the ITI is finished, a stimulus lamp is illuminated (**Stimulus phase**).
- If a correct reaction occurs during stimulus presentation, i.e. exhibits a correct nose-poke reaction - the lamp is switched off and **a reward is delivered into the stimulus hole**. The system now enters a **WAIT phase\***.
- If an incorrect reaction occurs the lamp is switched off – **no pellet is issued**. If defined, a **timeout period** follows. The system then enters the next **WAIT phase\***.
- If **no** reaction occurs in the Stimulus phase the **hold interval** follows – if defined.
- If a correct reaction occurs now the lamp is switched off and **a reward is delivered into the stimulus hole**. The system now enters the next **WAIT phase\***.
- If an incorrect reaction occurs the lamp is switched off – **no pellet is issued**. If defined, a **timeout period** follows. The system now enters the next **WAIT phase\***.
- If **no** reaction occurs in the hold interval the system enters the **WAIT phase\***.
- **\*WAIT phase:** the system waits for the animal to nose-poke into hole 6 in order to initiate the next ITI.
- Then the next stimulus cycle follows.
- If a hole is visited before stimulus presentation (premature reaction) in the ITI then this has no consequences either for the animal or for the trial run.
- No new stimulus will appear for as long as one of the light barriers is interrupted. The system waits until all light barriers are free again. Then the next trial begins.
- The experiment is finished after the predefined number of trials have been performed or the set maximum time has elapsed. The **total trial duration** is therefore variable as the stimulus phase and the hold interval length depend on the animal's reactions and may not reach the entered maximum values; the ITI can also be lengthened beyond the entered value by a continuous interruption of one of the light barriers



Trial Monitor Standard Paradigms  
 Shown is the display for a 1-Dispenser-Box

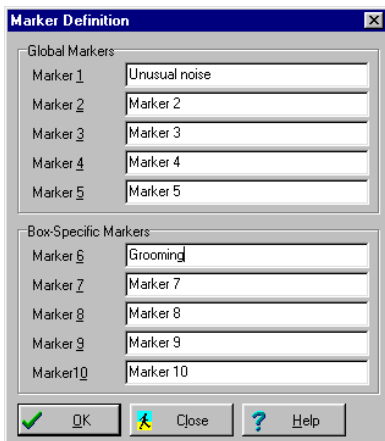


This is a 5-Dispenser-Box run in the 1-Dispenser-mode (the only active feeder here is the feeder mounted to the front wall)

When the experiment is finished in one box this does not affect data acquisition in other boxes. The test can also be terminated prematurely in a specific box any time.

### Event markers

**Event markers** can be entered via the keyboard in order to record any event that is of importance for the test procedure. They are assigned a text by the user and are added to the data file.



### Data Analysis

No.	Time	Event
1	0:00:00	Intertrial Interval
2	0:00:10	Wait for NP 6
3	0:00:36	Sensor 6 interrupted
4	0:00:36	Light 1 ON
5	0:00:41	Hold Interval
6	0:00:46	Intertrial Interval
7	0:00:56	Wait for NP 6
8	0:01:10	Sensor 6 interrupted
9	0:01:10	Light 1 ON
10	0:01:16	Hold Interval
11	0:01:18	Test End

The **run table** provides detailed information about the temporal course of the experiment. The table contains the following columns:

No.	Running number of the event
Time	Time elapsed from start of trial (h:m:s)
Event	Name of the event

**Markers** appear as event in a separate line.

In the **results table** the following parameters are calculated:

<b>No. of trials</b>	Total number of trials.
<b>No. of premature reactions</b>	Number of nose-poke reactions in the premature phase (can be defined by the operator)
<b>Trials w/o reactions</b>	Trials without reaction.
<b>Trials correct reactions</b>	Trials in which the animal has made a nose-poke reaction in the <i>stimulus</i> hole (during the stimulus phase or during the hold interval)
<b>Trials incorrect reactions</b>	Trials in which the animal has made a nose-poke reaction in an <i>incorrect</i> hole (during the stimulus phase or during the hold interval)
<b>Latency correct reactions</b>	Mean delay period from the start of the stimulus phase up to the occurrence of a correct nose-poke reaction (calculated using all correct nose-poke reactions)
<b>Latency incorrect reactions</b>	Mean delay period from the start of the stimulus phase up to the occurrence of an incorrect nose-poke reaction (calculated using all incorrect nose-poke reactions)
<b>Distribution hole 1...5</b>	Total number of visits in holes 1...5 (including visits in intertrial intervals)
<b>Only, if module 6 has been used:</b>	
<b>Nose-pokes in hole 6</b>	Total number of visits in hole 6
<b>Latency nose-pokes in hole 6</b>	Mean delay period from correct reaction up to next visit to hole 6

## Analysis - Results

5-Hole-Box / Key-OBS, Orleans, France (2001-990)

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Anim No.	3
Trial No.	1
Experiment No.	1
Operator	ES
Start	04.02.02 12:20 Duration 13 min
Comment	
Code	ABC
Strain	
Group	Control
Age	days
Weight	g
Substance	
Dose	
ITI	30 s
Stimulus Duration	5000 ms
Hold Interval	5 s
Timeout Interval	5 s
Premature Interval	5 s

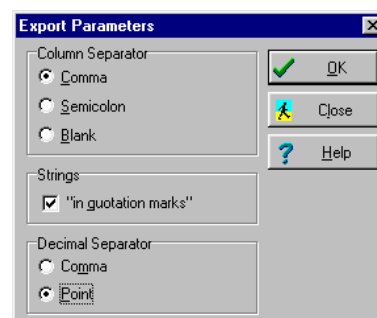
No. of trials	10
No. of premature reactions	1
Trials w/o reactions	1 = 10,0%
Trials correct reactions	5 = 50,0%
Trials incorrect reactions	4 = 40,0%
Latency correct reactions	2286 ms
Latency incorrect reactions	3415 ms
Distribution hole 1..5	4/12,6/1/1
Nose-pokes in hole 6	13
Latency nose-pokes in hole 6	43860 ms

New parameters are continuously added to this parameter list. If your paradigm requires specific calculations to be implemented please contact us!

## Data export

In order to use the trial results for further-reaching statistical calculations in statistics packages (e.g. SAS) or spread sheets (e.g. EXCEL) the system offers the possibility of storing data of the selected records in the form of **export files**.

Selectable column and decimal separators allow the adaptation of the export file to the particular import program used.



## Partial list of users

- Baylor College of Medicine, Houston, TX, USA
- Ecole Polytechnique Federale de Lausanne - EPFL, Lausanne, Switzerland
- Istituto Superiore di Sanita, Roma, Italy
- KEYOBS S.A., Orleans Cedex, France
- Leibniz-Institut für Neurobiologie, Magdeburg, Germany
- Max-Planck-Institut für Psychiatrie, München, Germany
- Medical Research Council Harwell - MRC, Oxfordshire, Great Britain
- National Defense Medical Center, Taipei, Taiwan
- National Institute of Health - NIH, Bethesda, MD, USA
- Universidade do Minho, Braga, Portugal
- University of Leicester, Leicester, Great Britain

## References

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- **Van Gaalen MM, Stenzel-Poore M, Holsboer F, Steckler T.** Reduced attention in mice overproducing corticotropin-releasing hormone. *Behavioural Brain Research* 2003; 142: 69-79.
- **Steckler T, Sauvage M, Holsboer F.** Glucocorticoid receptor impairment enhances impulsive responding in transgenic mice performing on a simultaneous visual discrimination task. *European Journal of Neuroscience* 2000; 12: 2559-69.



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