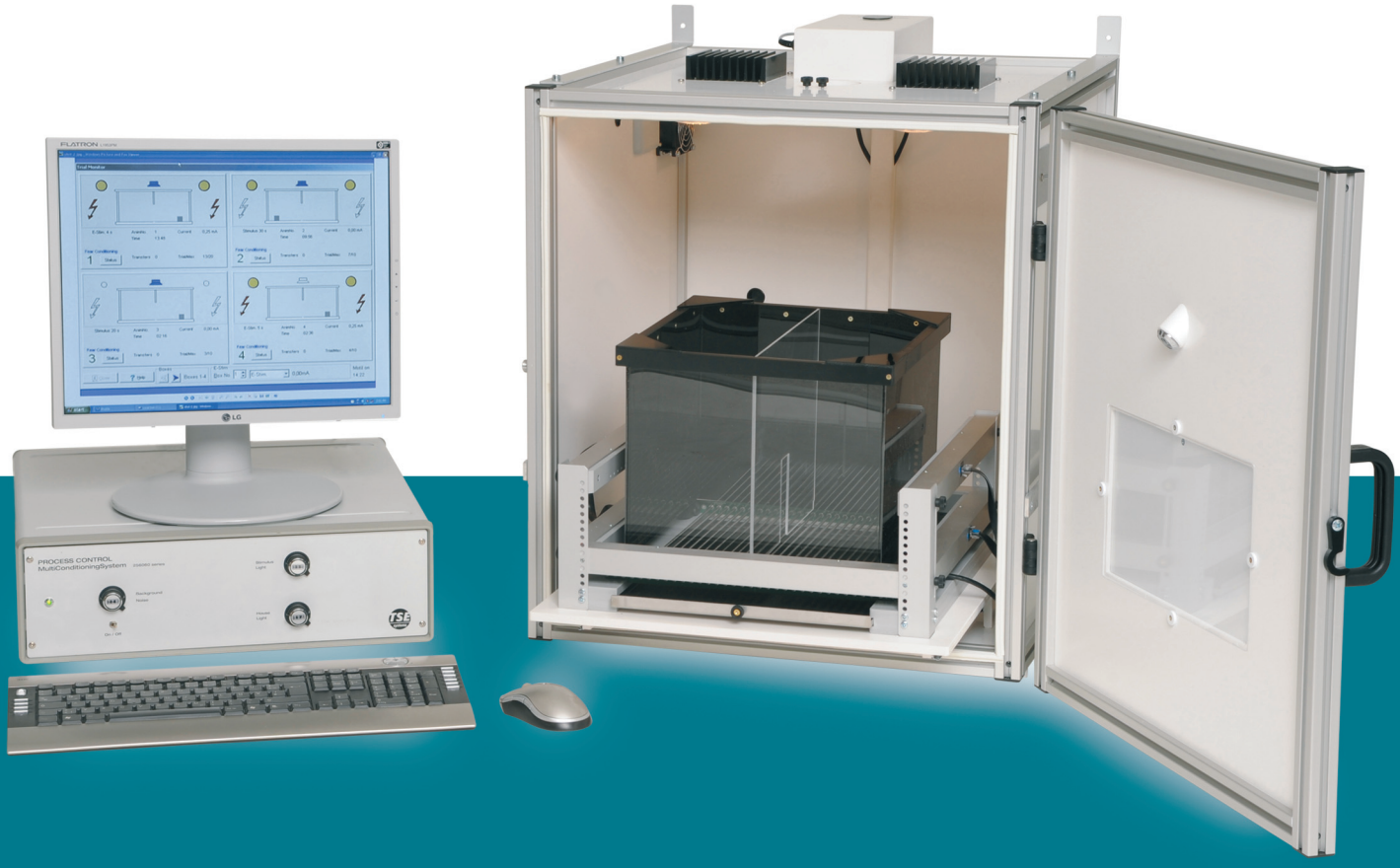
Sophisticated Life Science Research Instrumentation



TSE Multi Conditioning System

For mice, rats & other small laboratory animals

– Specifications subject to change without notice –

TSE Multi Conditioning System

„Learning is key“

When Charles Darwin formulated his Theory of Evolution in 1859, one of his central ideas was that the most adaptive species will best survive environmental changes. Applied on the individual organism that means that being optimally adaptive leads to evolutionary but also immediate benefits. The most powerful form of adaptiveness is adaptive behavior, i.e. **learning**.

There are many forms of learning, from simple learning like habituation and sensitization to complex learning which for example requires reasoning, but the most powerful and best investigated form of learning in animals is **conditioning**, especially Pavlovian conditioning - the learning of an association between a stimulus (the unconditional stimulus) with a particular biological meaning and another stimulus which did not have this meaning so far – the conditional stimulus.

Pavlovian conditioning experiments come in a variety of different tasks – each task specialized on specific aspects of learning and measuring a specific behavioral response. Intentions of such investigations can be to characterize a mouse or a rat strain, transgenic animals, effects of environmental stimuli, effects of a pharmacological treatment, manipulation of a particular brain site which is potentially involved in these forms of learning, and much more. In many neurodegenerative diseases multiple forms of learning are impaired.

Many of these forms of learning can be optimally investigated with the **TSE Multi Conditioning System**. This unique system is conceived as a modular integrated multi-purpose solution that allows the extensive testing on one single hardware and software platform. It is not only an economic alternative to individual specialized testing systems – the combination of different paradigms allows the design of completely novel experiments far ahead of present approaches.

A variety of hardware components is available to adjust the system to your experimental requirements. Select between different sensor frames each equipped with high-resolution light-beam sensors to pick up activity and animal movement. Choose the arena suited for your paradigm, adjust a variety of stimulus qualities – then select the appropriate software module and start your experiments. It´s as easy as that.

The following software modules to perform learning tasks are available to date:

- Fear Conditioning
- Passive Avoidance
- Active Avoidance
- Learned Helplessness
- Place Preference Conditioning

In addition to that unconditioned anxiety and general locomotor activity can be monitored with the

- Light-Dark Test Module resp.
- Activity Monitoring Module

Due to its flexibility and easy adaptability the **TSE Multi Conditioning System** is the system of choice for comprehensive animal testing!

The system is in continuous development in close cooperation with our users and new features are added frequently.

Please contact us to discuss your specific needs!

Features & Benefits

- High-resolution IR technology
- Sound stimuli - including ultrasound for rats / Light stimuli - variety of light qualities
- Electric shocks & air puffs - independent or simultaneous in both compartments
- Variable compartment design - tactile and/or visual discrimination
- Simultaneous video monitoring
- Versatile designing of experiments
- Combination systems for rats and mice
- Fully automated simultaneous operation in multiple chambers
- Software features in compliance with GLP requirements, e.g. audit trial

The whole is more than the sum of its components

Function Module Fear Conditioning

- Evaluate fear learning, retention and extinction
- Definition of experimental designs for trace & delay fear conditioning
- “New Context” experiments – adjustment of visual, auditory and tactile cues
- Optional ultra-sound loudspeakers for evaluation of panic responses in rats – unique in the market

The Fear Conditioning Module of the **TSE Multi Conditioning System** is designed to examine cued and contextual fear conditioning in rodents – one example of Pavlovian conditioning.

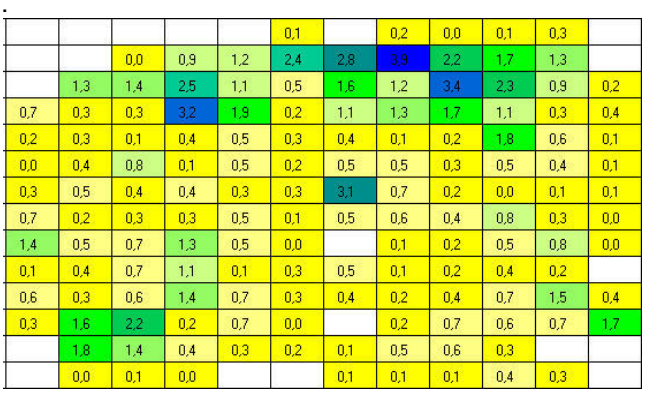
An unconditional fear stimulus like a foot shock induces fear behavior like the freezing response: the animals tend to remain in motionless, defensive posture. After several pairings of the foot shock with a neutral stimulus like a tone (training), the tone becomes a conditional fear stimulus which is now able to induce a freezing response. This behavior is evaluated in the subsequent retention trial - the animal is tested for its fear of both the context and/or the auditory cue.

Sine sound and light can be used as stimulus. Intensity of both stimuli as well as sine sound frequency are manually adjusted by the user.

The flexible FCS software module in the **TSE Multi Conditioning System** allows to create user-defined stimulus sequences for trace as well as delay fear conditioning protocols. The following stimuli are available:

- Stimulus sound
- Stimulus light – software-adjustable frequency
- Electric Shock – constant or pulsed, software-adjustable amplitude

Black arenas and grid cover plates are available to modify the context in the retention test. In addition to that background noise and house-light intensities can be adjusted to increase difference between training and retention context (multisensory cues).

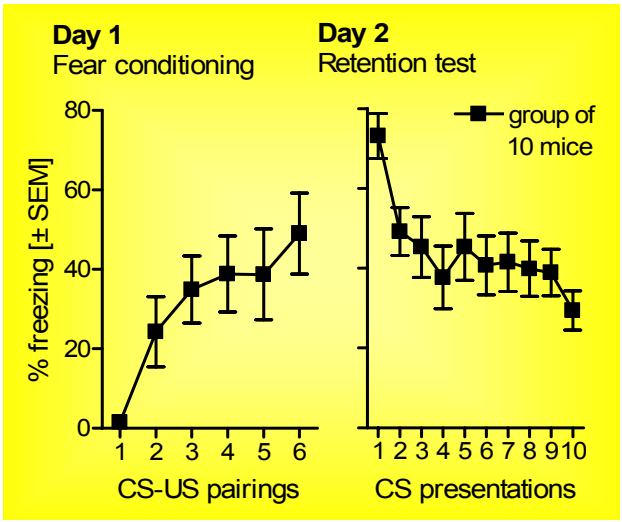


					0,1		0,2	0,0	0,1	0,3	
		0,0	0,9	1,2	2,4	2,8	3,9	2,2	1,7	1,3	
	1,3	1,4	2,5	1,1	0,5	1,6	1,2	3,4	2,3	0,9	0,2
0,7	0,3	0,3	3,2	1,9	0,2	1,1	1,3	1,7	1,1	0,3	0,4
0,2	0,3	0,1	0,4	0,5	0,3	0,4	0,1	0,2	1,8	0,6	0,1
0,0	0,4	0,8	0,1	0,5	0,2	0,5	0,5	0,3	0,5	0,4	0,1
0,3	0,5	0,4	0,4	0,3	0,3	3,1	0,7	0,2	0,0	0,1	0,1
0,7	0,2	0,3	0,3	0,5	0,1	0,5	0,6	0,4	0,8	0,3	0,0
1,4	0,5	0,7	1,3	0,5	0,0		0,1	0,2	0,5	0,8	0,0
0,1	0,4	0,7	1,1	0,1	0,3	0,5	0,1	0,2	0,4	0,2	
0,6	0,3	0,6	1,4	0,7	0,3	0,4	0,2	0,4	0,7	1,5	0,4
0,3	1,6	2,2	0,2	0,7	0,0		0,2	0,7	0,6	0,7	1,7
	1,8	1,4	0,4	0,3	0,2	0,1	0,5	0,6	0,3		
	0,0	0,1	0,0			0,1	0,1	0,1	0,4	0,3	

Spacial Analysis

The analysis of the FCS software module can be configured to output

- a chronological list of freezing events
- a spacial graph showing the exploratory behaviour of the animal in the arena
- a table with calculated parameters incl. the number & duration of freezing events, distance travelled, number of jumps and rearing events, average speed, activity times and more…



The classical parameter to characterize the animal’s fear response over the course of the experiment is the % freezing time – the data above show results of a typical experiment in mice.

For further processing of the results with the aid of complex statistical programs all the calculated parameters can be stored in user-defined export files.

Function Module Passive Avoidance

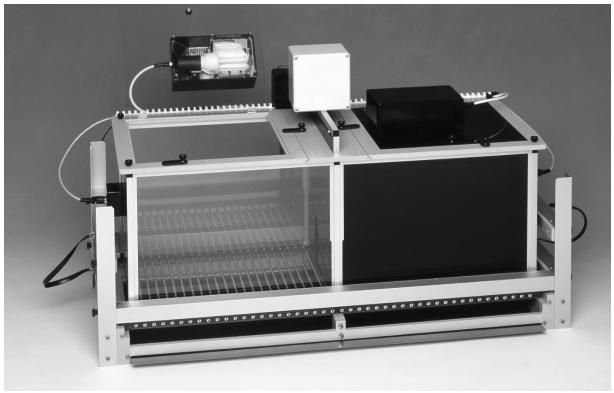
- Evaluate associative emotional learning
- Definition of experimental designs for pre-exposure, training and retention
- High-resolution activity data for a new quality of behavior assessment

The passive avoidance task is a behavioral paradigm based on associative emotional learning. It combines Pavlovian conditioning with the expression of an instrumental response and is therefore more complex than the behavior shown during fear conditioning.

In the memory test (retention) the animal makes a choice by either avoiding or entering a dark compartment in which it has experienced an aversive foot shock during the previous training session.

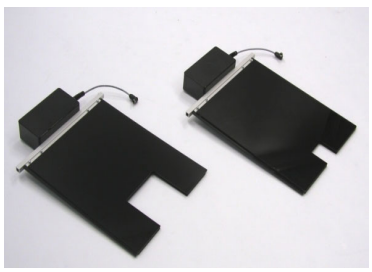
A pre-exposure session is used to habituate the animal to the arena before the training is performed.

The latency to refrain from crossing into the punished compartment during the retention test serves as an index of the ability to avoid, and therefore allows memory to be assessed.

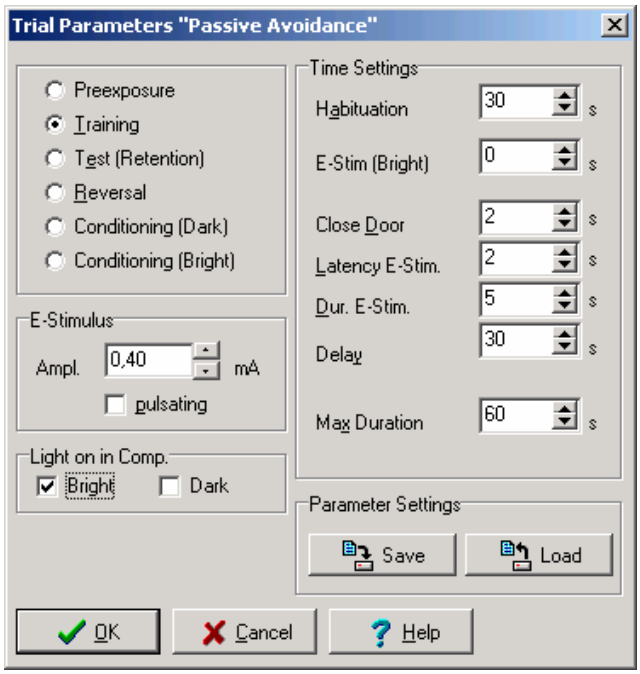


Large frame

The MCS system offers special light dark arenas that are equipped with a motorized door.



For a high level of aversiveness of the bright compartment special high-intensity lamps are used – that are mounted either to the housing ceiling if camera observation is to be done in parallel or mounted in lids that are placed on the arena – this depends on your system configuration.



Control Parameters

The Passive Avoidance module of the MCS software allows the easy definition of all control parameters required to run a typical PA protocol.

The analysis generates several tables calculating among others the step-through latency – this is the classical measure for memory performance – and a variety of activity based parameters. This new approach that is exclusively available in the **TSE Multi Conditoning System** adds a new quality in evaluation of the animal´s movement characteristics especially inside the dark compartment (shock response).

		Bright Compartment					Dark Compartment				
Phase	Duration	Vmean (cm/s)	Inact. (%)	Expl. (%)	Rear. No.	Jump No.	Vmean (cm/s)	Inact. (%)	Expl. (%)	Rear. No.	Jump No.
Habituation	00:30:0	17,7	33,8	56,4	0	0	--	--	--	--	--
Door open	00:30:0	5,5	73,1	17,1	1	0	0,0	0,0	0,0	0	0
	00:30:0	4,6	22,7	18,6	0	0	13,2	7,0	39,3	0	0
	00:30:0	5,7	18,9	21,4	0	0	10,5	17,8	35,7	1	0
	00:30:0	3,3	2,6	14,3	0	0	14,7	31,1	50,0	0	0
	00:30:0	4,5	13,0	24,3	0	0	11,6	23,2	37,1	1	0
	00:30:0	7,6	22,9	32,9	0	0	7,1	12,8	20,0	0	0
	00:30:0	0,6	4,9	5,0	0	0	13,1	30,8	37,9	0	0
	00:30:0	6,6	30,7	27,9	1	0	5,4	23,2	17,1	0	0
	00:30:0	0,0	0,0	0,0	0	0	12,9	42,0	25,7	1	0
	00:30:0	0,0	0,0	0,0	0	0	8,0	62,5	15,0	0	0
	00:30:0	6,7	25,4	25,7	0	0	5,4	17,5	19,3	0	0
	00:30:0	2,5	5,3	13,6	0	0	7,2	51,6	15,7	0	0
	00:30:0	5,3	32,5	22,1	1	0	6,1	24,6	27,9	0	0
	00:30:0	0,0	0,0	0,0	0	0	7,1	64,0	22,1	0	0

Function Module Active Avoidance

- Bidirectional avoidance conditioning (“Step-Through Avoidance”)
- Select between software-controlled light, sine sound, electric shock and tactile stimulus (air puff)
- Optional application of WAV files for acoustic discrimination
- Flexible program editor for generation of user-defined procedures

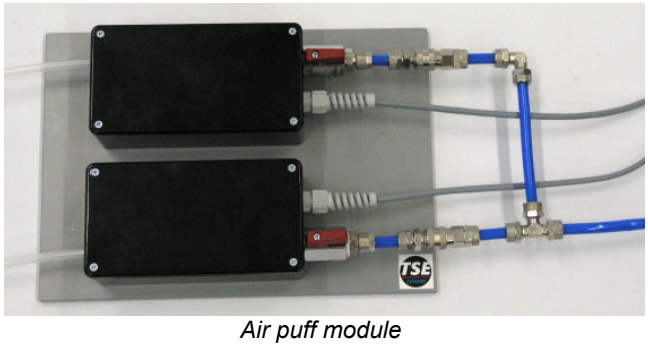
The active avoidance paradigm is a fear-motivated associative avoidance test that provides an easy way to evaluate associative learning and memory in rodents.

The task is designed to test the ability of the animal to avoid an aversive event such as a foot shock by learning to perform a specific behavior – in this case the change into the other side of the 2-compartment arena – in response to a cue – the conditioned stimulus.

The measures recorded, mainly

- number of avoidances: the animal crossing to the other compartment during the stimulus signal
- number of non-responses: the animal failing to cross to the other compartment during the trial
- response latency: latency to avoid or escape

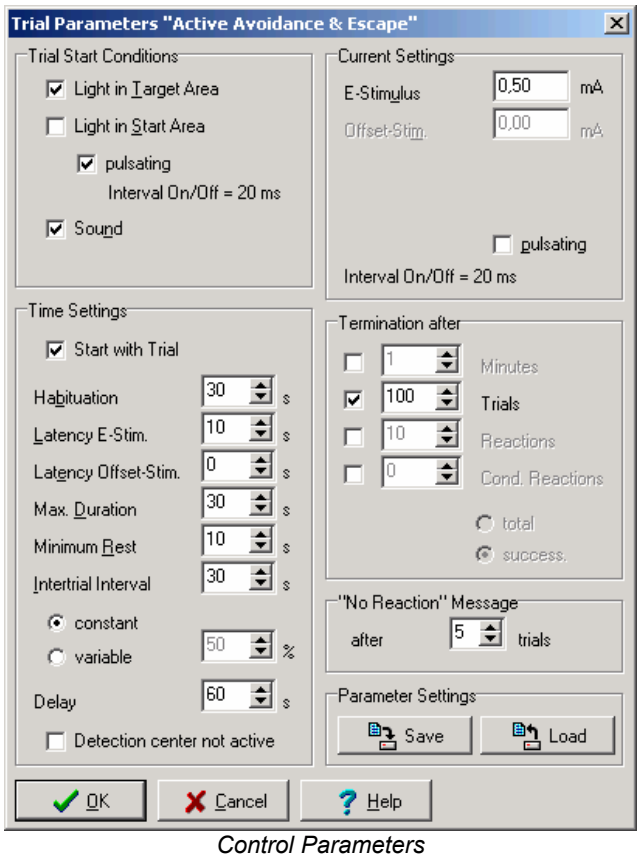
serve as an index of learning and allow memory to be assessed.



Air puff module

The experiment is usually run in a clear acrylic 2-compartment arena. Automatic doors are not required for the task.

Electric hurdles as compartment dividers are available as an option that may facilitate animal reaction especially in mice.



Control Parameters

The Active Avoidance software module of the **TSE Multi Conditioning System** is designed to run a variety of test protocols. Adjustable time parameters include habituation, time to shock, shock length, maximum run duration and inter-stimulus interval.

Data analysis provides a results table listing conditioned, unconditioned and inter-stimulus transfers and mean reaction times. Distance traveled is presented as a measure of activity throughout the trial. Measured values of several animals can be summarized for group analysis. Freezing events can be added if required – an exclusive feature of the **TSE Multi Conditioning System.**

Trials	100
Cond. Reaction (Avoidance)	80
Uncond. Reaction (Escape)	20
No Reaction (Escape Failure)	0
Transfers Habituation	6
Intertrial Transfers	52
Transfers Delay	1
Transfers Total (w/o Habi)	153
Transfers Total (with Habi)	159
Mean Latency CR	3,85 s
Mean Latency UR	10,61 s
Mean Latency all Trials	5,20 s

Results Table “Active Avoidance”

Function Module Learned Helplessness

- Well-known model for depression – test based on active avoidance performance
- 2-compartment test boxes with electrified floor – hurdle optional
- Optional use of cue light & sound
- Various shock characteristics - including adjustable pulse frequency and shock application pattern
- Dedicated software modules for inescapable shock session and subsequent active avoidance task

Learned Helplessness is a well-known animal model for depression.

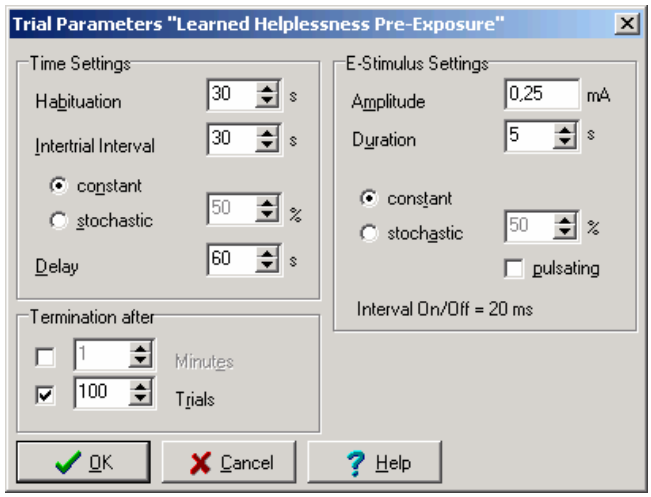
Exposure of animals to an unpredictable, inescapable moderate foot shock will induce a curious phenomenon: animals learn to ***not*** respond with a possible useful response in the subsequent shock escape test. These response deficits in the subsequent learning task are called "helpless" behavior. They may improve during treatment with antidepressant drugs.

LHN test box shown in a MEDIUM sized frame

During the inescapable shock session unpredictable foot shocks are applied in both compartments simultaneously.

In the subsequent escape trial the animal can terminate the shock by transferring to the other compartment (2-way active avoidance task). Learning deficits can be clearly seen by the reduced number of escape reactions. The number of inter-trial transfers and distance travelled can be presented as a measure of activity – select among a variety of analysis parameters! Analyse animals individually – or perform a group analysis for easy performance comparisons!

In the Learned Helplessness module of the **TSE Multi Conditioning System** predefined software modules for both conditioning ("Pre-Exposure") and testing – this is a classical Active Avoidance procedure – are provided ready-to-use.



Control Parameters

If your system configuration includes 3-dimensional light beams with high resolution the parallel measurement of the animal's freezing behavior for studying fear responses in addition to helpless behavior is made possible – the seconds spent freezing between each pair of CS-footshock presentations is calculated – an exclusive feature of the **TSE Multi Conditioning System.**

Transfers Habituation	2
Transfers Shock	25
Transfers Intertrial	11
Transfers Delay	0
Sum Transfers (w/o Habi)	36
Sum Transfers (with Habi)	38

Results Table "LHN Pre-Exposure" - Example

SNo.	Trials	Habi	CR	UR	NoR	ITI	TT(woH)
1	100	0	61	39	0	102	206
2	100	6	80	20	0	52	153

SNo.	TT(wH)	Lat.CR(s)	Lat.UR(s)	Lat.AT(s)
1	206	4,07	10,43	6,55
2	159	3,85	10,61	5,20

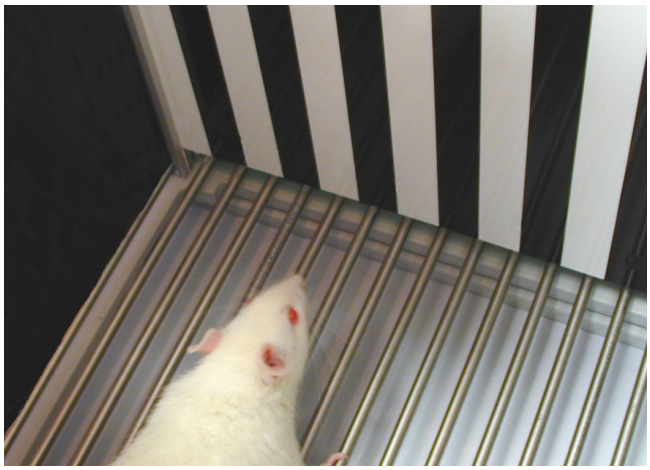
Group Table "LHN Test (=AA)" - Example

Function Module Place Preference

- Assess the rewarding properties of a psychotropic drug
- Test boxes with a selection of wall colors and patterns
- Floor inserts to facilitate discrimination between compartments (tactile cues)
- Compartment dividers: Closed dividers, tunnel gates, or software-controlled doors
- High-resolution infrared sensors monitor entries into compartments and locomotor activity

Conditioned place preference is a widely used technique to assess the rewarding properties of a psychotropic drug. Treatment with a specific preparation is repeatedly paired with a distinct environment while a control treatment is paired with a different environment. When the animal has access to both environments, preference for the drug-paired cues, i.e. increased time of stay in the drug-paired compartment, will indicate the rewarding effects of the test drug.

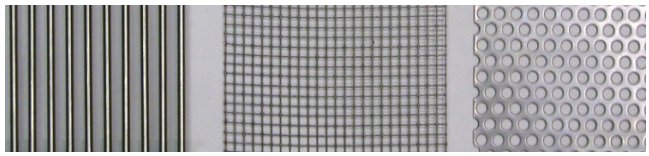
Learning processes like those observed in conditioned place preference are believed to be involved in the development of addiction.



The Place Preference function module available for the **TSE Multi Conditioning System** requires a special Place Preference insert to be placed inside the light-beam frame.

This insert is a 2-compartment arena providing visual cues through differently patterened/colored walls (standard: vertical pattern of black/white stripes vs. transparent acrylic plastic or with a black or white coating) and as an option differently structured floor inserts to facilitate tactile discrimination what is especially useful for mice.

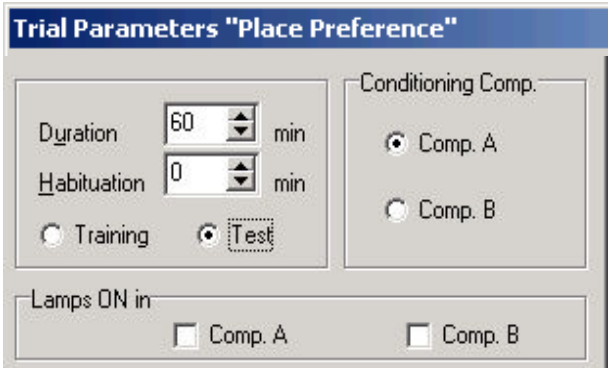
Both compartments are divided by a closed wall made of clear or black acrylic to restrict access of the animal to one compartment during **conditioning**. For the **test** this closed wall is exchanged for a divider with a gate of animal-specific size. In a system with automatic doors handling is much more comfortable – the doors are operated by the software depending on the experimental phase selected.



Example of tactile floor inserts

In the system configuration with lamps mounted to the box lids these software-controlled lamps can be used to counterbalance a natural preference of the animal to a specific compartment.

The Place Preference function module available for the **TSE Multi Conditioning System** allows to select between training and test sessions.



Control Parameters

Evaluation of the test trial includes:

- number of visits to each compartment
- latency first transfer to the other compartment
- time of stay: absolute & percentage for each compartment
- distance travelled: absolute & percentage for each compartment
- number of rearings for each compartments
- average speed

Due to the high-resolution locomotory information and the ability of the system to measure rearing behavior much more data is available about potential sedative effects of a specific treatment – not available in standard systems that rely on transfer detection only – another advantage of the **TSE Multi Conditioning System**.

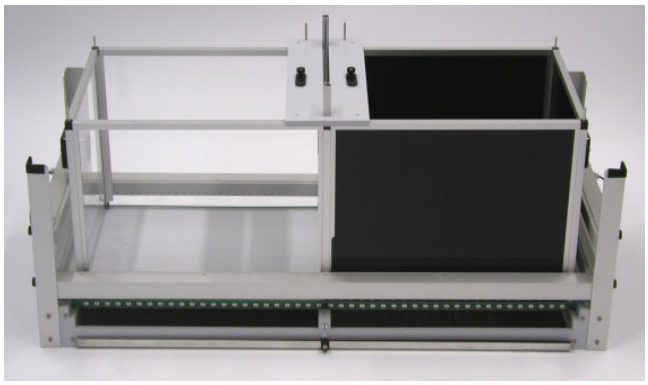
Function Module Light-Dark Test

- Quick and easy test on unconditioned anxiety – no training required
- 2-compartment arenas with 50% dark area
- Floor grid cover plates to modify tactile cues
- Can be run with or without automated doors

The light-dark paradigm is a quick and easy-to-use test to evaluate the unconditioned anxiety level of animals, i.e. it does not require prior training of the animal.

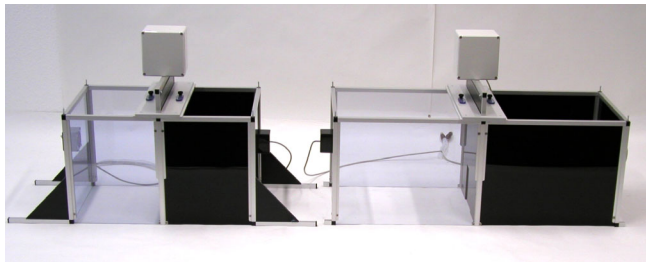
It is based on a conflict between the innate aversion to brightly illuminated areas and the spontaneous exploratory behavior in response to a novel environment. If given a choice between a brightly illuminated aversive compartment versus a dark compartment (safe area), rodents spontaneously prefer the dark.

The Light-Dark test has been added to the **TSE Multi Conditioning System** to allow the evaluation of general effects on emotions during complex conditioning testing. It complements the FCS module in that it can be used to check whether fear conditoning has changed the unconditioned anxiety level of the animal. It also allows to test the effect of test compounds: Anxiolytic compounds decrease the total duration of time spent in the dark compartment (index of aversion) and the number of transfers between compartments (index of activity-exploration) while anxiogenic compounds work in the opposite way.



LARGE frame with LD Arena Rat (shown withoud lid and without automatic door)

The function module Light-Dark Test available for the **TSE Multi Conditioning System** requires a special light-dark insert to be placed into the light-beam frame. If your system configuration already includes a Passive Avoidance arena this can be used for the Light-Dark test without further modification. This 2-compartment insert has a dark compartment that comprises 50% of the arena area and it is equipped with a central tunnel gate to let the animal pass. An automated door is available as an option but is not required to run the protocol.



Size comparison mouse & rat inserts configured for the LARGE frame

The floor grid is usually covered by a plastic cover plate to faciliate animal movement.

If your system is equipped with high-intensity lamps available in the housing or – as an option – mounted to the arena lids very aversive conditions up to 700 lux can be created on the bright side of the arena.

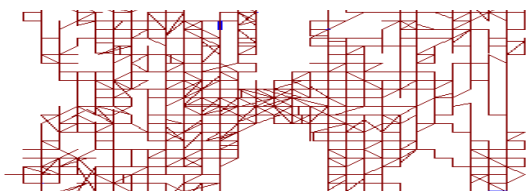
Evaluation of the test includes a table listing the following parameters for each compartment:

- number of visits
- latency first visit
- time of stay

Anim.	Date	Start	Light	Dark	Time L	Time D	Latency
1	05.09.2007	10:29:00	2	1	00:24:6	00:05:9	00:23:5
3	05.09.2007	10:42:40	1	1	00:52:4	00:27:6	00:27:6
3	06.05.2008	15:20:59	0	1	00:00:0	01:20:0	01:20:0
43	06.05.2008	15:25:36	0	1	00:00:0	01:20:0	01:20:0
43	06.05.2008	15:43:58	0	1	00:00:0	01:20:0	01:20:0

Many more parameters are available as an optional upgrade to the standard table – whatever is required for your experiments.

Due to the high-resolution infrared sensors a detailed look into the locomotor behavior of the animal throughout the task is possible in parallel. An example is the pattern of movement that can be generated with a keystroke.



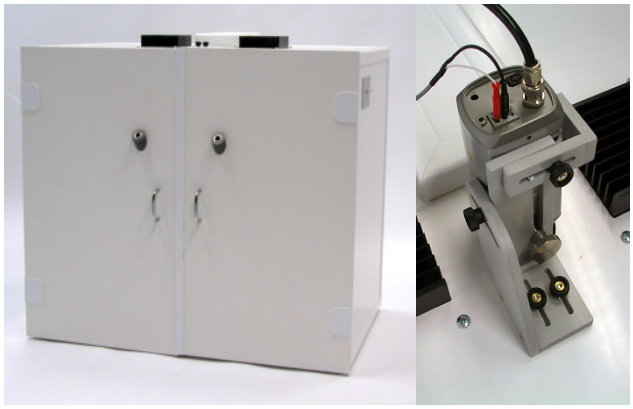
Pattern of Movement

Function Module Activity

- General evaluation of 3-dimensional locomotory data
- Graphical and numerical display of the animal´s movement throughout the arena
- Global and phase-related analyses
- Optional infra-red illumination for additional camera observation even in total darkness

A behavioral response in a conditioning experiment can be attenuated since a treatment induces sedation but not learning deficits. Therefore, a 'must' in behavioral experiments is to control for more general effects of an experimental manipulation.

With the function module Activity of the **TSE Multi Conditioning System**, general effects on locomotor activity (e.g., exploration, spatial patterns) can be easily obtained. These experiments can be performed independent from the conditioning experiment – if required existing conditioning data can be reevaluated by using the analysis algorithms of this module.



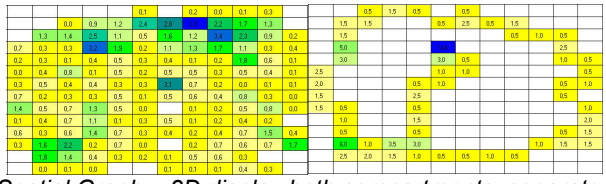
Left: MCS Housing – a controlled environment
Right: Optional camera

For independent measurements any type of arena provided for the **TSE Multi Conditioning System** can be used. The floor grid can be covered by a plastic plate if required (for longer lasting trials the use of the floor grid is recommended). Special arenas with a fixed floor are available that allow the use of bedding for longer lasting experiments.

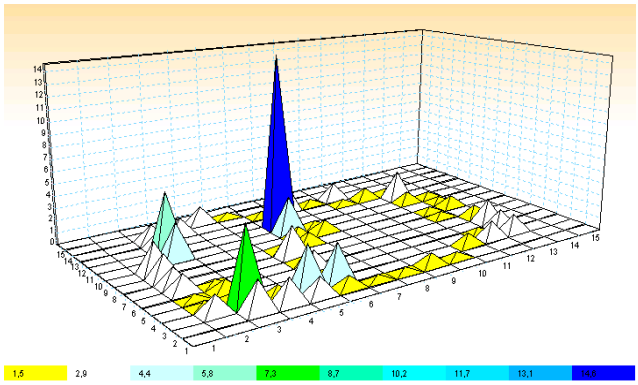
Grid cover plate

Adjust the background noise level and house light illumination to meet your requirements. Software-controlled lamps can be used to switch the illumination on and off at specified time points.

The movement of the animal can be displayed graphically in a pattern of movement and a spacial graph. The spacial graph shows the temporal distribution of the animal´s stay in the box as well as any exploratory preferences.



Spatial Graph – 2D display both compartments, separated by a divider)



Spatial Graph – 3D display (one compartment, i.e 50% of the arena area)

A variety of locomotory parameters are provided for each compartment:

- Distance travelled
- No. of rearings
- Rearing Time %
- No. of jumps
- Resting Time absolute and in %
- Activity Time absolute and in %
- Hyperactivity Time %
- Exploratory area covered in % of the total box area
- Mean speed

Taken together these additional analysis features add a new dimension to any learning experiment – making the **TSE Multi Conditioning System** to the ideal tool for all complex animal testing procedures.

Sophisticated Life Science Research Instrumentation

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. It is our corporate goal to become the number one manufacturer of highly sophisticated products in each market we serve.

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