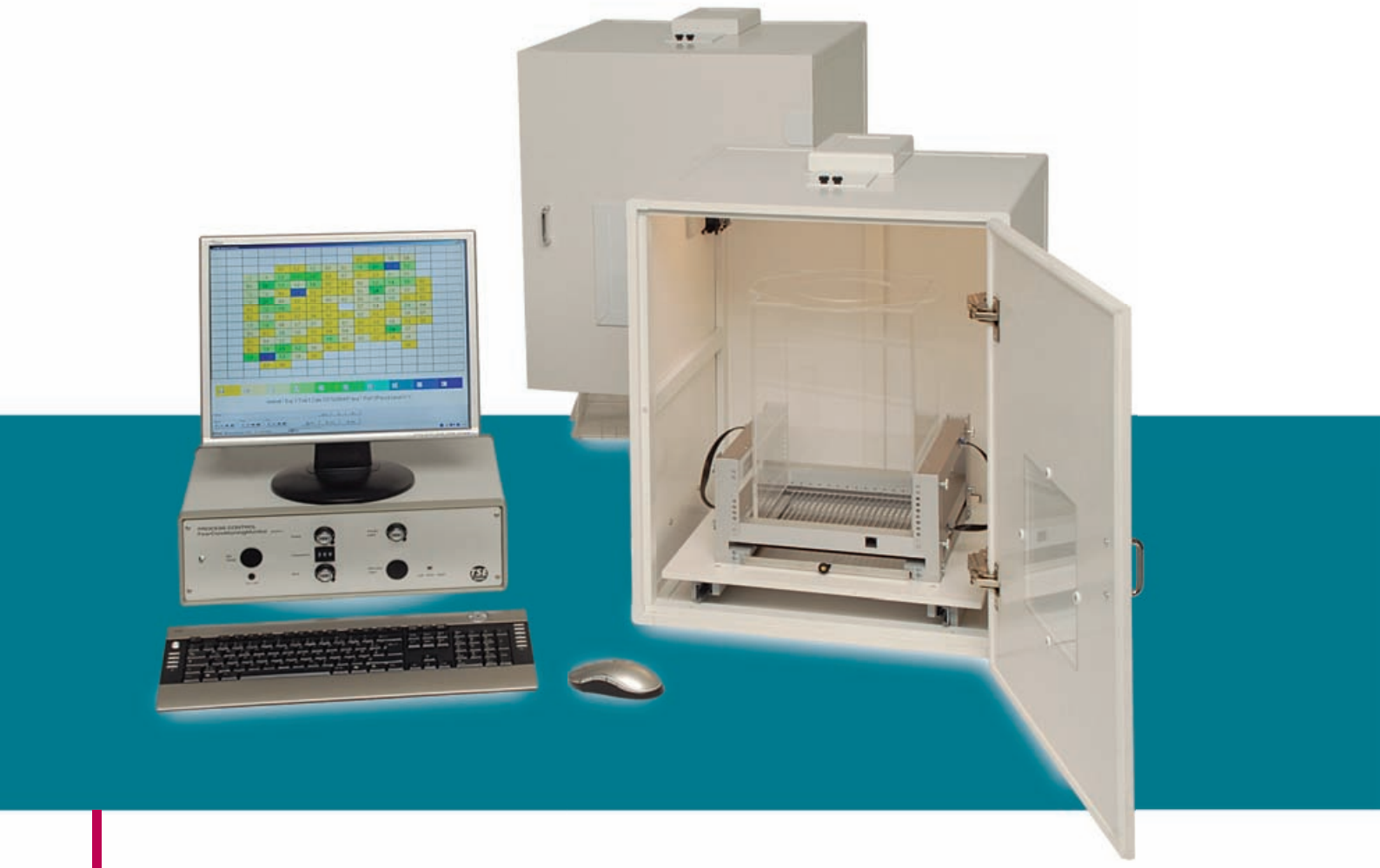
Sophisticated Life Science Research Instrumentation



Fear Conditioning System

For small laboratory animals

– Specifications subject to change without notice –

TSE Fear Conditioning System

FCS - Configuration SMALL ADVANCED for mice

The system

The **TSE Fear Conditioning System** (FCS) has been developed to study **contextual** and **cued fear** in rodents (mice or rats).

In classical **fear conditioning**, a behavioral paradigm based on associative emotional learning, the subject is given a cue (*conditioned stimulus*), usually an auditory signal, followed by a short electric shock (*unconditioned stimulus*) Alternatively only an electric shock can be given (cued vs. contextual fear conditioning). This is called the **conditioning trial**.

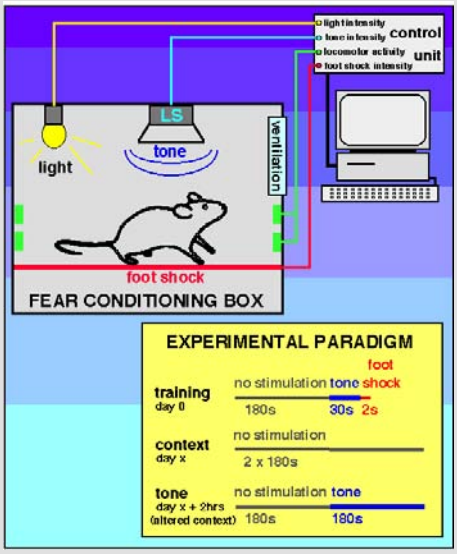
The animal can subsequently be tested for its fear to both the context and the auditory cue. Shock induced fear is expressed as freezing: the animals tend to remain in motionless, defensive posture (behavioral inhibition). Normal rodents usually exhibit a clear conditioned fear response, i.e. freezing, to *both* the context and the tone in a different context. This **retention test** can be conducted immediately after the training or some days later and can thus give information about short-term and long-term memory influences on conditioning.

The **TSE Fear Conditioning System** is a widely used and proven system that allows a comprehensive analysis of fear learning & extinction. In addition to evaluating freezing frequency and duration it offers a variety of other evaluation parameters for a detailed characterization of the fear behavior over the course of the experiment. It is a valuable tool to learn how information about aversive emotional experiences is processed and stored in the brain, it can be used to evaluate the cellular basis of fear learning and extinction and it allows to study the underlying mechanisms of the effect of anxiolytic drugs.

The system can also be converted into a light-dark testing system to perform unconditioned anxiety tests in addition to fear conditioning experiments. The integrated ultra-sound function can be used to evaluate panic responses in rats and place preference arenas are available to run CPP tests. A large variety of optional components and accessories allow to adjust the system to the individual requirements.

The system is continuously further developed in close cooperation with our users. If you are interested in a specific modification contact us!

Example of a Fear Conditioning experiment



Schematic representation
of the fear conditioning paradigm

In the following example the test consists of 3 phases:

FCS Control File

Phase 1
- Part 1 Pause 180 s
- Part 2 Sound 70 ms 30 s
- Part 3 E-Stim 0,45 mA constant 2s
- Part 4 Pause 30 s

Phase 2
- Part 1 Pause 180 s

Phase 3
- Part 1 Pause 180 s
- Part 2 Sound 70 ms 180 s

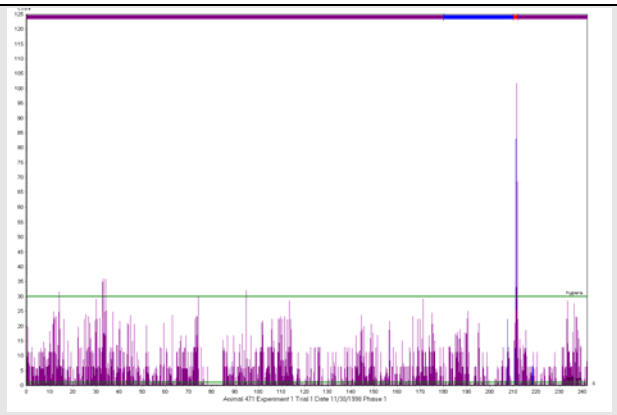
The sequence of stimulus presentation is determined by the operator.

- **Phase 1** represents the training trial on day1, where the animal is conditioned to the sound (CS).
- **Phase 2** (day2) is conducted with the animal placed into the conditioning context to evaluate contextual fear conditioning…
- …whereas in **phase 3** (day2 + 2hrs) the context is altered to monitor auditory (cued) fear conditioning.

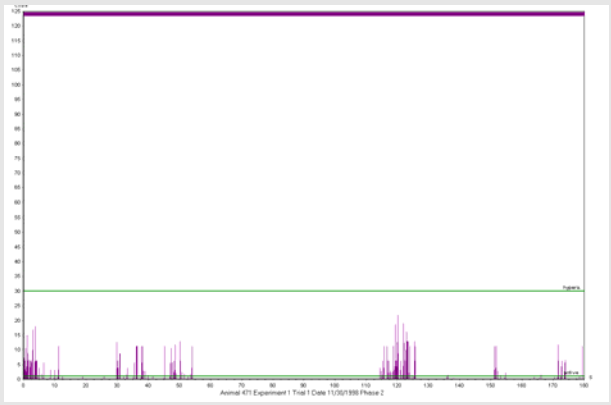
The **activity patterns** clearly show the animal´s contextual fear during phase 2 of the experiment, where no stimulus is given. The same animal doesn´t show any freezing reaction – displayed here as reduced activity - after altering the context (phase 3, part 1). In contrast in the auditory cue test (phase 3, part 2) an obvious fear reaction can be observed.

The behavior displayed in the graphs is also shown in the results table. A high number of freezing events (column **Fre**) and a drastically elevated total freezing duration (column **FreD**) in the conditioning context (phase 2 – Pause) is visible. The same is true for the cue phase ("Sound") in the new context.

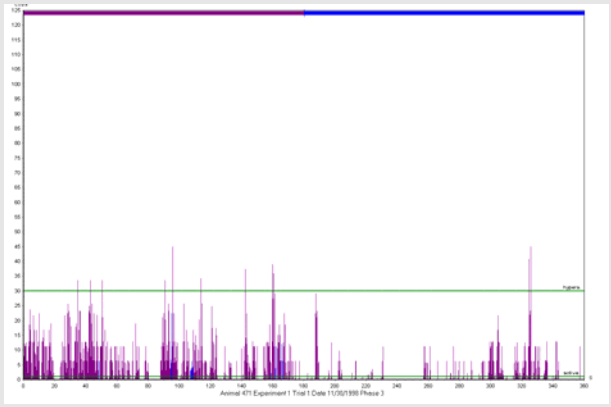
Please note that the threshold for activity has been set very low in the example (1cm/sec)



Training DAY1 – Conditioning Context



Conditioning Context DAY2



Altered Context/Sound DAY2 + 2hrs

Phase	Part	Dis.	Rear	%	XY	%	Gro	Man	Fre	FreD	Jum	Act	%	Hyp	%
1	Pause	1137.3	0	0.0	1	100.0	0	0	1	7	0	25	87.7	5	0.3
	Sound	149.3	1	0.4	2	99.6	0	0	1	2	0	7	74.3	0	0.0
	E-Stim	64.5	2	18.1	3	78.7	0	0	0	0	1	1	78.0	2	41.6
	Pause	157.7	2	0.7	3	99.3	0	0	0	0	0	8	78.0	0	0.0
2	Pause	199.9	0	0.0	1	100.0	0	0	11	116	0	20	22.0	0	0.0
3	Pause	858.3	10	3.3	10	96.6	0	0	9	33	1	40	68.6	9	0.5
	Sound	262.6	0	0.0	1	100.0	0	0	21	99	0	38	29.1	2	0.1

Phase	Part	Dis.	Vmean	Vmax	VSD	VSEM	Rest	%	Expl%
1	Pause	1137.3	6.32	36.1	30.45	0.23	22.2	12.3	44.0
	Sound	149.3	4.98	25.1	26.46	0.48	7.7	25.7	12.9
	E-Stim	64.5	32.20	101.8	164.21	11.61	0.4	21.0	5.8
	Pause	157.7	5.26	28.4	26.76	0.43	6.6	22.0	15.6
2	Pause	199.9	1.11	21.8	11.52	0.09	140.4	78.0	7.6
3	Pause	858.3	4.77	45.0	26.53	0.20	56.6	31.4	58.2
	Sound	262.6	1.46	44.9	13.45	0.10	127.6	70.9	19.1

Results Table

Besides the freezing behavior the animal shows decreased exploratory behavior in both the context and during the cue (column **Expl%** - the percentage of area explored by the animal). Time spent active **(Act%)** and mean speed (**Vmean**) are reduced as well.

System components

The TSE Fear Conditioning System consists of the following components:

- **FCS measuring units -** including a so-called ***test box*** and a special ***housing***
- **Control unit** incl. **PCI-interface** for IBM-compatible computer
- **Options** and **accessories**
- **Fear Conditioning Software** for Windows.

Up to 4 measuring units can be monitored with 1 PC in the standard setup.

An extension for a maximum number of 8 units is possible.

2 different configurations of the TSE Fear Conditioning System are available:

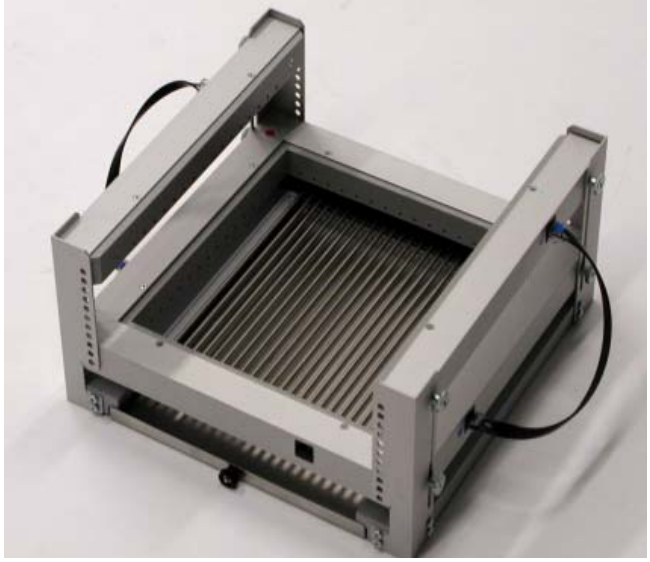
- configuration **SMALL ADVANCED** for mice
- configuration **LARGE ADVANCED** for rats & mice

The test box

A test box comprises:

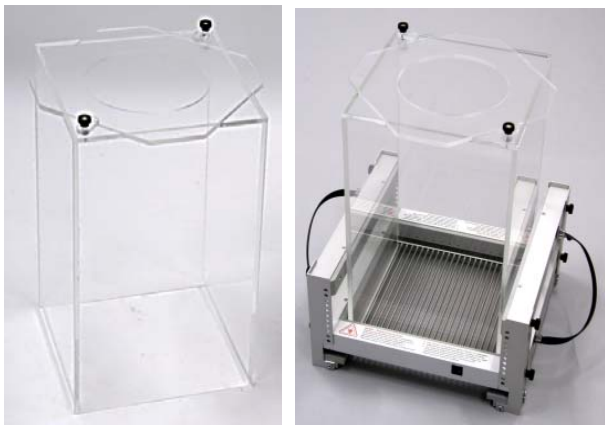
- **Base construction** ("frame"), square-shaped, with integrated animal detection sensors
- Removable **drop pan** (stainless steel; matt finish to prevent reflections)
- Removable stainless steel **foot shock grid** connected to a shocker-scrambler unit for delivering shocks of defined duration and intensity
- **Perspex Arena** - placed inside the sensor frame
- **PVC plates** that can be placed on the shock grid to modify the context

◆ ***Configuration SMALL ADVANCED***



This system has a small frame suitable for mice only.

- **Sensor frame**: XY and Z axis featuring **16** sensors mounted **14 mm** apart. Size (outer): 310x310 mm (WxD).
- **Grid:** Floor bars 4 mm diameter, distance rod center to rod center 8.9 mm.



- **Perspex Arena**: 230x230x350 mm with removable lid with central cut-out.

◆ ***Configuration LARGE ADVANCED***

This system features a large sensor frame that can be used for rats and mice.

- **Sensor frame**: XY and Z axis featuring **32** sensors mounted **14 mm** apart. Size (outer): 540x540 mm (WxD).

2 different types of floor grids are available (mouse and rat specification) that can be easily exchanged. Both have to be ordered separately.

- **Grid *Rat*:** Floor bars 6 mm diameter, distance rod center to rod center 19.5 mm.
- **Grid *Mouse*:** Floor bars 4 mm diameter, distance rod center to rod center 8.9 mm.

The standard arena is a large perspex arena suitable for rats and mice.

- **Perspex Arena *Large***: 460x460x470 mm with removable lid with central cut-out.

If a smaller exploratory area is required for mice a so-called ***template*** can be placed onto the floor grid. This template holds a small acrylic arena in place.

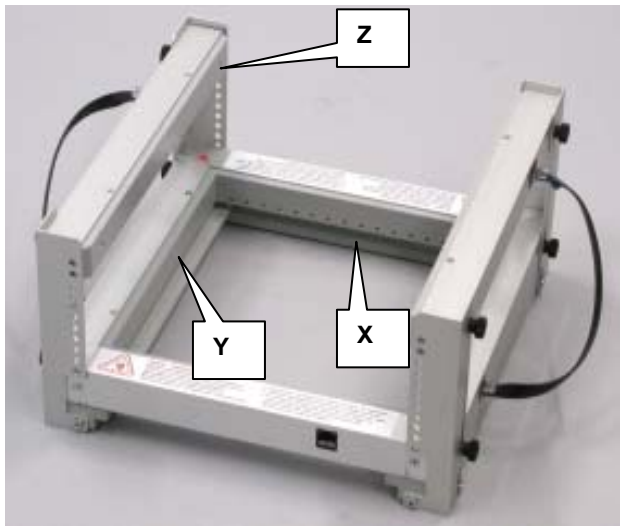
- **Perspex Arena *Small***: 230x230x350 mm with removable lid with central cut-out.

The location sensors

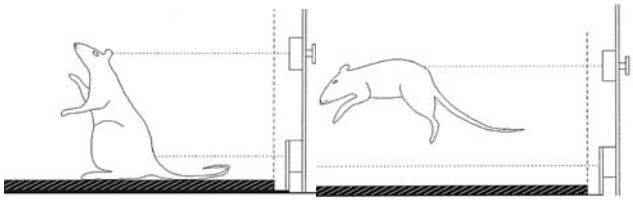
The animal´s position and movement inside the arena are monitored with the help of **infra-red light-barriers** mounted into the frame.

Each single light-barrier consists of one transmitting and one receiving LED. The light emitted by the transmitter is invisible to the animal.

The so-called **X-Y level** with two pairs of light-barrier strips arranged at right angles to each other in the same plane is used to determine the horizontal co-ordinates of the animal and thus its location.



The second sensor level is the so-called **Z-level** that allows to detect rearing and jumps, i.e. vertical activity. The Z strips are height-adjustable to meet your individual requirements.

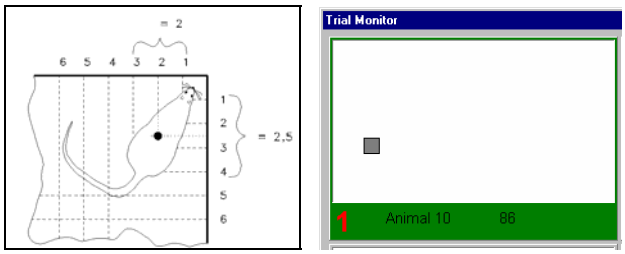


Rearing
XY+Z interrupted

Jump
Only Z interrupted

All sensors are scanned with a sampling rate of **up to 100Hz**, i.e. the position of the animal is checked up to 100 times a second! In combination with the extraordinarily high density of the infra-red sensors (spacing 14mm!) this results in a high spatial and temporal resolution making the system able to reliably detect very small and very short movements.

As the body of the animal normally covers several light barriers in each axis, an **averaging** procedure is used to define the coordinates of its „centre of gravity“.



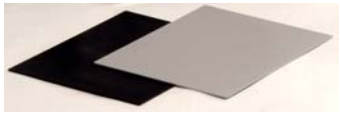
Averaging method

As a result of this averaging method the number of possible of „animal positions“ is **doubled** when compared with the actual density of the light barrier grid.

The coordinates of the center of gravity of the animal are stored for analysis.

Box accessories

In both configurations – LARGE and SMALL - the standard clear arena comes complete with 2 floor plates made from PVC. These plates can placed on the grid in order to modify the context (tactile cue).



Configuration SMALL ADVANCED

The housing

The test box is operated in a sound attenuating housing, featuring:

- A **ventilation fan** in the side wall
- A sliding **floor plate** for easy removal of test box
- A **loudspeaker** in the ceiling for software-controlled application of acoustic stimulus and/or background noise (up to 90 dB resp. 100 dB).

Instead of the standard loudspeaker integrated in the SMALL housing (2…20kHz) a special ultrasound loudspeaker is built into the LARGE housing suited to generate sound signals up to **25 kHz**. This extended range allows for the first time to evaluate panic responses in rats as a reaction to 22 kHz sine sound (see page 17)!

Configuration LARGE ADVANCED

Configuration SMALL ADVANCED

SMALL ADVANCED

LARGE ADVANCED

- **2 lamps** (SMALL) resp. **4 lamps** (LARGE) in the ceiling for continuous house light illumination (software-controlled).
- a **hinged door.**

The LARGE housing has a double door and is equipped with a peep hole as standard (a window is available on request).

The SMALL housing has a single hinged door that is equipped with a viewing window (a peep-hole is available as an option) that can be closed.

Housing dimensions:

SMALL	520 x 520 x 650 mm (WxDxH)
LARGE	730 x 730 x 830 mm (WxDxH)

Control unit & interface

The sensor frames as well as all the function elements of the housing are connected to a so-called control unit, configured for parallel control of 1, 2 or 4* measuring units.

An extension for a maximum number of 8 units is possible.

It features:

- A **sound & noise generator** for generating the auditory stimulus (sine sound, max. **90dB**) and white noise (max. **100dB**). Standard sine frequency range is **2-20 kHz** for the SMALL and **2-25 kHz** for the LARGE system. The sine sound can be continuous or pulsed - the pulse frequency is set by the operator.
- Power supply for **house light**: max. 200 Lux for the SMALL and max. 100 Lux for the LARGE. Left and right side of the illumination can be switched on and off independently from each other.
- **Knobs** on the front panel to adjust the amplitude of sound and noise as well as the light intensity.
- A **sine sound frequency** digital switch for adjusting the sound frequency in steps of 100Hz.
- A **shocker/scrambler**: This is a microprocessor controlled constant current generator with integrated current flow detector for pole reversal of the grid rods. The amplitude is software-adjustable in steps of 0.1mA up to 3.1mA. Alternatively pulsating stimulus current can be chosen.
- A **TTL output socket** (BNC) on the rear panel that outputs a TTL signal during the experiment (TTL high at start/ TTL low at end). This signal can be used to trigger a custom system, such as a telemetry or electrophysiology data acquisition system or the TSE Video-Kit (see **Options**).

The FCS control interface (PCI slot type) is designed to be built into an IBM-compatible computer with the Windows operating system (2000, XP, VISTA). The control unit is connected to this interface.

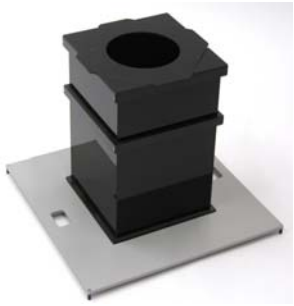
We can supply you with a complete system including the computer completely installed and ready for use!

Options

◆ ***"New context" arenas***

In order to further change the context special arenas made from black IR permeable acrylic are available. All arenas feature a black lid with central cut-out.

- The large black arena in the LARGE system has a fixed floor made from black PVC. A gray PVC plate is provided as well that can be placed inside the arena.



- The small black arenas in the SMALL and the LARGE configuration do not have a fixed floor. They can be combined with the floor grid or the PVC floor plates provided with the transparent arenas. In the LARGE system the SMALL arena has to be used in conjunction with a template.

◆ ***Option: High-intensity stimulus light***

The standard housing light is suitable for continuous background light only. For giving intense light stimuli the housing ceiling can be equipped with additional high-intensity LEDs (up to 700 Lux). They are used in the experiment as software-controlled light stimulus in parallel or instead of the acoustic stimulus. A switch

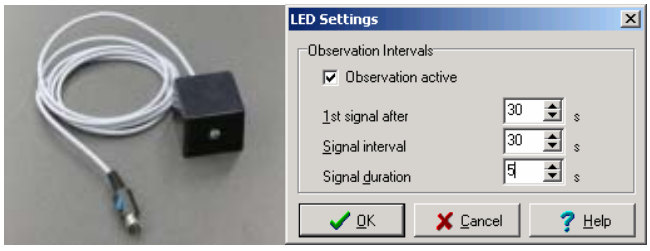
on the control unit allows the left-hand and right-hand lamp to be switched independently from each other. The house light is operated manually if stimulus LEDs are present (toggle switch mounted to the housing side).

◆ *Option: Stimulus plus background noise*

An additional noise channel in the control unit allows the application of white noise as a stimulus signal with continuous background noise given in parallel. The stimulus noise is software-controlled, continuous background noise is manually switched on and off at the control unit front panel in this configuration. The noise intensity is adjustable independently in both noise channels (identical for all connected boxes, max. 100dB).

◆ *Option: LED unit*

An external signal light, the so-called **LED unit** is available that is connected to the housing and that can be operated via the software. This light is used to signal a time window where behaviors of the animal (usually freezing) are to be recorded manually by the user.



◆ *Option: Video-kit*

If the experiment is to be recorded, **a camera/recorder combination** is available, the so-called "Video-Kit". For each measuring unit 1 kit is required.

The video is displayed on a connected monitor (not included in the kit). Recording is triggered using a **TTL signal** from the FCS control unit that is output when the experiment is started. By this means the behavior of the animal at specific time points and the numerical parameters calculated by the software - such as freezing - can be easily compared with each other.

◆ *Option: Trigger package*

This option extends the control unit by a **multi-trigger output**, with the help of which information about the status of the experiment can be transferred to an external data acquisition system (e.g. electrophysiology) in real time. A total of 6 signals is available in 6 separate channels: Experiment ON, Experiment OFF, Noise/Sound ON, Noise/Sound OFF, Shock ON, Shock OFF. Start and finish of each part appear in different output channels - this prevents misinterpretation of the information.

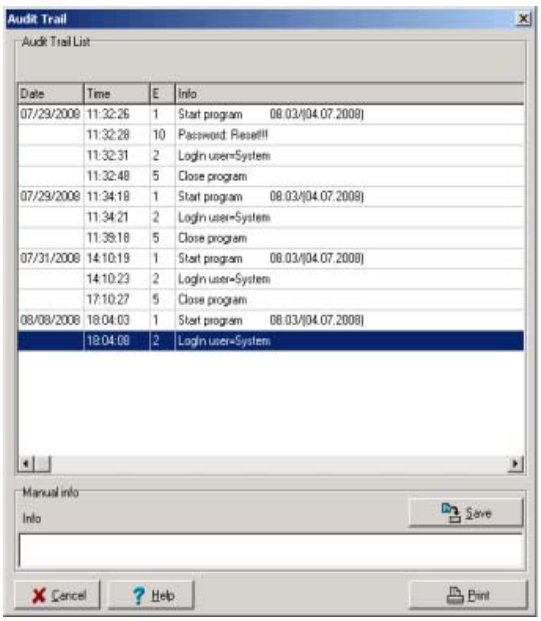
◆ *Other options*

- The housing door in the SMALL system can be equipped with a peep hole in addition to the observation window.
- The housing can also be colored dark in the interior. This reduces the aversiveness. In a system with several measuring units differently colored housings can be used for Training and Retention tests. The dark color then serves as an altered context.
- In contrast to the standard shocker (max. amplitude 3.1mA, 0.1mA steps), the maximum current strengths can be reduced down to 1.55mA as an option. Steps of 0.05mA are possible then.
- The desired frequency for the sine sound is usually selected with a 3-digit digital switch on the control unit front panel. An additional switch can be added to the control unit that can be used to switch to a fixed frequency extremely rapidly. Standard frequency: 10 kHz. If a different frequency is required (2...20 kHz SMALL resp. 2…25 kHz LARGE) please mention the frequency in your order.
- We also provide rectangular sensor frames on request (arena size 350x200 mm). Please contact us for details.

The FCS software

The software controls the test in the boxes, collects, displays and stores all experimental data and allows for detailed analysis and documentation. The user-friendly design of the software has been created to support the following GLP requirements:

- Protection against subsequent manipulation of raw data through special data file format
- Integrated user administration with login with a unique combination of user ID and password
- Automatic audit trail generation – date, time and user-stamped. Access and changes to the FCS software are logged in the audit trail

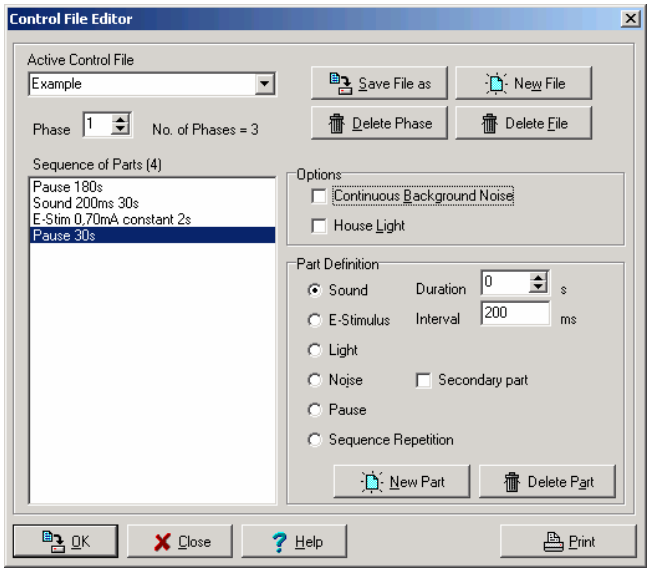


Defining the experiment structure

During the experiment a sequence of stimuli is presented in all active boxes simultaneously. This sequence is called the „**experimental structure**“. It is defined via a **control file** that is created in the so-called “Control File Editor”, a flexible tool that allows the free definition of stimulus sequences in trace and delay conditioning procedures.

You can define as many control files as are required and simply load the desired file before the experiment is started!

- With a **new** control file you always have to start definition with phase number 1.
- If the current file already consists of **more than 1 phase** select a number >1 in order to perform the desired changes (adding new parts, deleting existing parts).



Phase 1

- Part definition is performed in 3 steps:

  1. Select the part **type** by clicking on the corresponding radio button, e.g. Sound.
  2. Select the parameters available for this type, e.g. duration 30 seconds.
  3. If parallel operation of signals is required (i.e. sound and shock given simultaneously) activate the function “Secondary part”.
  4. Confirm by clicking on the button **New Part**.

- Now the next part can be defined. The list already defined is shown. During the experiment these parts are executed one after the other.

Depending on the hardware configuration the following parts are available:

Part Type	Parameters
Sound	**Stimulus sound** Duration: min. 1 second Interval: 100 ... 1000 ms The interval defines the pulse frequency of the sound signal (interval 100ms = sound frequency of 5Hz). If a **Zero** is entered than continuous sound is generated.
E-Stimulus	**Foot shock** Duration: min. 1 second Current strength: 0,1 ... 3,1 mA Current type: constant or pulsed
Light	**Stimulus light** Duration: min. 1 second *Only available if the system is equipped with high-intensity stimulus light*
Noise	**Stimulus noise** Duration: min. 1 second Interval: 100 ... 1000 ms The interval defines the pulse frequency of the

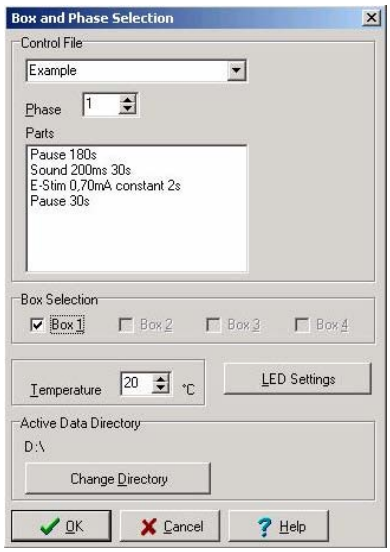
	noise signal (interval 100ms = frequency of 5Hz). If a **Zero** is entered than continuous noise is generated. *In a system with only 1 noise channel no continuous background noise can be applied in the experiment when noise is used as a stimulus.*
Pause	Duration: min. 1 second In a pause no signal is given.

- Each phase can consist of **up to 200 parts** – this means even very complex sequences - such as long-term extinction studies - can be defined.
- Complete part sequences can be repeated as often as is required ("Sequence Repetition").
- Up to 30 phases can be defined per control file. The experimental data of all these phases will be stored in one data file what is especially comfortable for long-term experiments because it makes the analysis of such experiments easier.

Usually **continuous background noise** is output during the experiment. This is done software-controlled in the setup with 1 noise channel and manually in the setup where a second noise channel is available for applying stimulus noise and back-ground noise in parallel.

Continuous **house light** can be switched on and off by the software. If 2 light channels are present in the system – house light plus stimulus light – house light is switched on and off manually – stimulus light is software-controlled in this case.

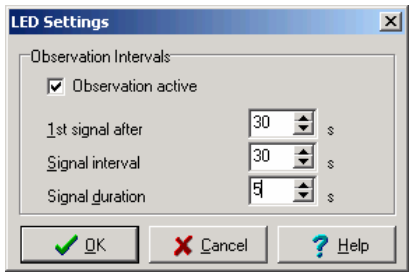
Performing a trial



⇒ Load the **control file** to be used,
⇒ Select the **phase number** – the contents of the phase is shown,
⇒ Activate the **boxes** to be included in the trial,
⇒ Enter the **temperature** - if desired,
⇒ Configure the **observational LED** and
⇒ Select the **directory** for data storage.

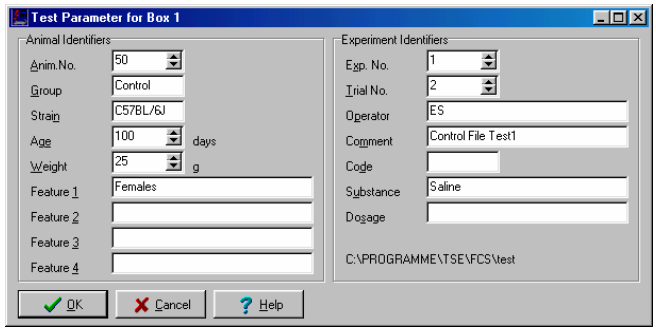
If the **LED unit** is included in the system it can be used to signal a time window where behaviors of the animal are to be recorded manually. The following parameters can be set:

- Time between phase start and appearance of first signal (**1st signal after**).
- **Interval** between signals.
- Signal **duration.**



A new experiment (phase 1)

Selecting **Phase 1** will start a **new** experiment, entering a number larger than **1** will allow to start a follow-up trial.



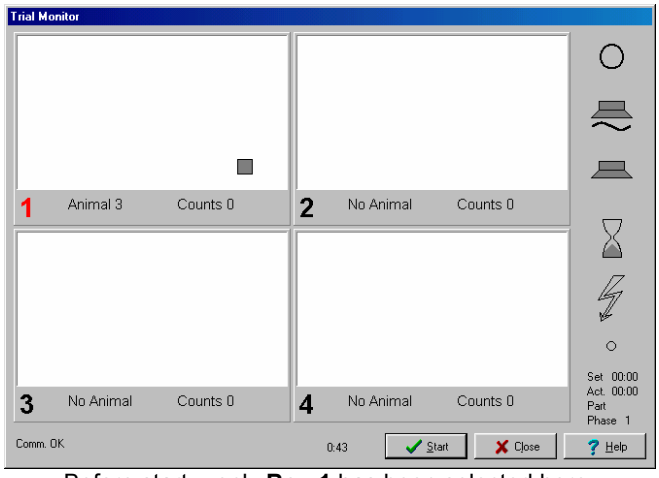
In Phase 1 the "Test parameters" window opens in which all the parameters used to describe the animal and the trial are defined. These entries are valid for all phases and any subsequent changes are prevented by the system!

Follow-up trial (phases 2 ... 30)

Entering a phase number **>1** will open a window where all data records available for a follow-up trial with this phase number are listed for selection. An existing record can now be selected for the next phase.

Starting a test

The ***trial monitor*** is then displayed, showing all 4 boxes simultaneously. The boxes prepared for an experiment are in the READY condition (red number). **House light** as stimulus - as well as **background noise** are switched on (if activated and available as software-controlled signal).



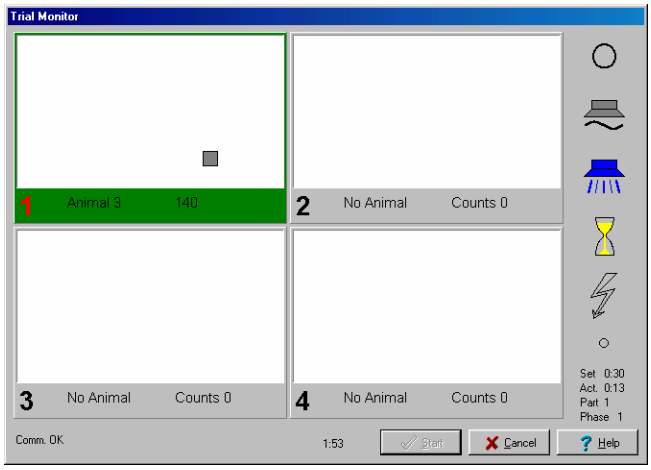
Before start - only **Box 1** has been selected here

Now the animals have to be placed into the boxes and the housings closed.

The test is started in all boxes <u>simultaneously</u> with the button START.

The running experiment

During the experiment the animal´s position inside the box is continuously displayed with a moving symbol. This square corresponds to the coordinates of the calculated centre of gravity. If the animal rears (presence in XY and Z) this square turns blue.



Graphical symbols represent the active part (light, noise, sine sound, E-stimulus, pause). An active LED is symbolized by a green circle.

Status information is added:

- total time to end of current part (**Set**)
- time already elapsed after start (**Act**)
- the current part number (**Part**)
- in the active **phase**
- the number of moves, i.e. interruptions of light barriers (**Counts**)

The system automatically records all animal position coordinates for subsequent analysis.

Manual recording of behavioral events

In addition to detecting freezing behavior automatically according to the parameter „Freezing“ (***Analysis Parameters***) the user can observe the animal and record freezing behavior manually at any time. One key on the keyboard is reserved per box. In the results table manually recorded freezing events are output in a separate column (**Man**). Usually the external LED unit is used to signal the time window when manual recording of freezing is to be performed.

5 additional keys on the keyboard are available for Box 1 for manual registration of other behavioral events such as grooming, rearing etc. The information is output in the raw data table, in the activity graph and in a separate behavior table.

Test end

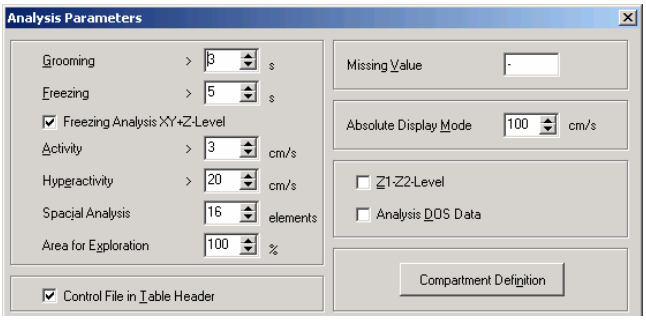
When all parts of the chosen phase have been executed a test is ended **automatically** in all boxes simultaneously. The user can also terminate the test **manually** any time.

The system prevents the program being terminated inadvertently when boxes are still active. This procedure is in accordance with the provisions of the Good Laboratory Practice code (GLP).

Analysis of measured data

After the experiment has been finished the measured data can be analyzed. A variety of graphs and numerical tables are provided.

In the menu item ***Analysis Parameters*** the definition of parameters which influence the presentation of the analysis data is possible.



The most important parameters are the **threshold settings:**

Grooming (in seconds)
Time threshold for automatically recorded "grooming" behavior. Grooming is interpreted whenever a **rearing event** (=sensor levels XY+Z interrupted) has exceeded this duration.

Freezing (in seconds)
Time threshold for automatically recorded "freezing" behavior. Freezing is interpreted whenever the animal **hasn´t been moving** for more than this duration, i.e. no change in sensor status has been detected in X, Y and Z.

Activity (in cm/sec)
Speed threshold for calculating „activity" frequency and duration. Activity times include hyperactivity times. This threshold will also be seen in the activity graph as a horizontal line.

Hyperactivity (in cm/sec)
Speed threshold for calculating „hyperactivity" frequency and duration. This threshold will also be seen in the activity graph as a horizontal line.

◆ *The raw data table*

Phase 1 23.02.2001 10:44
Pause Dur. 30s Values 234

Time (ms)	X(cm)	Y(cm)	Z
0	9,4	7,8	0
2810	8,5	7,8	0
2890	9,4	7,8	0
3070	8,5	7,8	0
3150	8,5	7,3	0
3680	8,5	7,8	0
3810	8,5	8,6	0
4130	9,4	8,6	0
4150	10,8	8,6	0
4330	10,8	7,8	0
4530	11,8	7,8	0
4700	11,8	8,6	0
4860	13,2	8,6	0

With the menu item ***Raw Data Table*** the animal´s position coordinates are listed in detail.

A new line is only then written into this file whenever the center of gravity has changed its position or the status of the Z level has been altered. If the position has not changed then *no* line is stored. The raw data table is therefore discontinuous. This avoids storage of redundant data if the test animal remains motionless (i.e. reduction of the memory space).

- The **phases** are output one after the other with their number, start date and start time.

- The **parts** of each phase are listed chronologically with their total duration and the number of entries, i.e. lines in the table.

The table has the following columns:

- The first column represents the **time** in **ms** from the start of *each phase*. With every new phase the time column is restarted.

- The next 2 columns is the **X-** and **Y-coordinate** of the centre of gravity converted into **cm**.

- The column „Z" gives information on the status of the unidimensional rearing indicator **Z**:

  ⇒ **0**=Z not interrupted, i.e. animal in XY-level
  ⇒ **1**=XY+Z interrupted, i.e. animal is rearing
  ⇒ **2**=only Z, but not XY interrupted, i.e. the animal is jumping.

- An additional column „**Status**" describes events such as

  ⇒ **"rearings"** (detection in XY **and** Z),
  ⇒ **"jumps"** (detection in Z only)
  ⇒ **"no animal"** (no sensors interrupted)
  ⇒ Manually entered freezing events for Box1...4
  ⇒ Marker keys **1...5** for Box1 given with start time and duration (in ms)

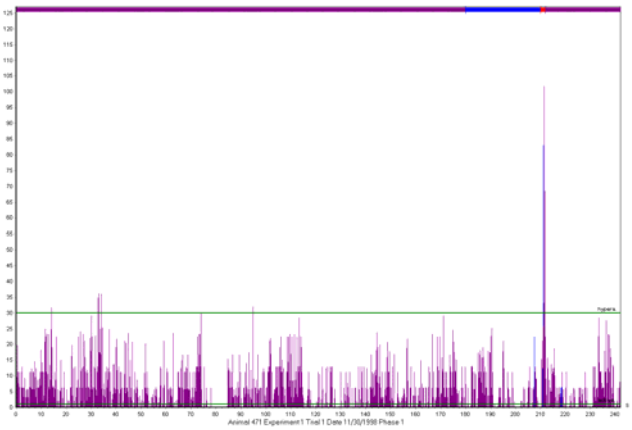
◆ *The activity graph*

The activity graph is an overview about the animal´s activity. It displays one phase with all its parts of a specific animal. If more than 1 phase is available in the file these phases can be called up one after another.

A colored bar at the upper screen indicates the different parts of the phase:

violet	Pause
blue	Sound/noise stimulus
yellow	Light stimulus
red	E-stimulus

Measure of activity is the **animal´s momentary speed**. The coordinate system shows the **time** in seconds as the X-axis and the **speed** in cm/sec as the Y-axis. The momentary speed is displayed as a **vertical line**.



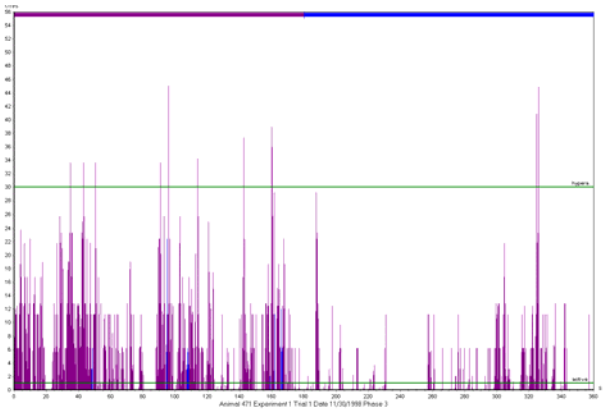
The color of the speed lines has the following meaning:

violet	Detection only in XY-level
blue	Rearing (detection in XY and Z)
yellow	Jump (detection only in Z)

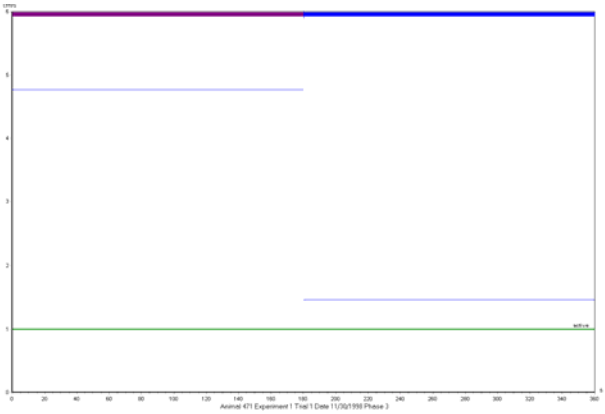
The current thresholds for activity and hyperactivity are shown as green horizontal lines allowing a quick overview about the speed distribution during the experiment.

The *relative* presentation is the default setting when the graph is activated. The display can be switched to the *absolute* presentation. Absolute presentation with a standardized Y-scale makes it easier to compare data of different experiments.

The momentary speed display can be switched to show the **mean speed per part**.



Momentary speed (relative representation)



Mean speed (relative representation)

If behavioral markers have been entered during data acquisition for box 1 they appear als horizontal colored lines below the phase bar. Each of the 5 different markers is output in its own color. Beginning and end of the behavior is indicated.

Manually entered freezing events are symbolized by vertical gray lines below the phase bar. Each single line represents one event.

The graph can be saved as is displayed as BMP-file.

◆ *The table of results*

The ***table of results*** lists a series of parameters calculated from the raw data. The most important parameters here are the number of freezing events with the total freezing duration, the mean speed and total distance travelled, the time spent active and the exploratory area.

Phase	Part	Dis	Rear	%	XY	%	Gro	Man	Fre	FreD	Jum	Act	%	Hyp	%
1	Pause	1137,3	0	0,0	1	100,0	0	0	1	7	0	25	87,7	5	0,3
	Sound	149,3	1	0,4	2	99,6	0	0	1	2	0	7	74,3	0	0,0
	E-Stim	64,5	2	18,1	3	78,7	0	0	0	0	1	1	79,0	2	41,6
	Pause	157,7	2	0,7	3	99,3	0	0	0	0	0	8	78,0	0	0,0
2	Pause	199,9	0	0,0	1	100,0	0	0	0	11	116	0	20	22,0	0	0,0
3	Pause	858,3	10	3,3	10	96,6	0	0	0	9	33	1	40	68,6	9	0,5
	Sound	262,6	0	0,0	1	100,0	0	0	0	21	99	0	38	29,1	2	0,1

Phase	Part	Dis.	Vmean	Vmax	VSD	VSEM	Resl	%	Expl%
1	Pause	1137,3	6,32	36,1	30,45	0,23	22,2	12,3	44,0
	Sound	149,3	4,98	25,1	26,46	0,48	7,7	25,7	12,9
	E-Stim	64,5	32,20	101,8	164,21	11,61	0,4	21,0	5,8
	Pause	157,7	5,26	28,4	26,76	0,49	8,6	22,0	15,6
2	Pause	199,9	1,11	21,8	11,52	0,09	140,4	78,0	7,6
3	Pause	858,3	4,77	45,0	26,53	0,20	56,6	31,4	58,2
	Sound	262,6	1,46	44,9	13,45	0,10	127,6	70,9	19,1

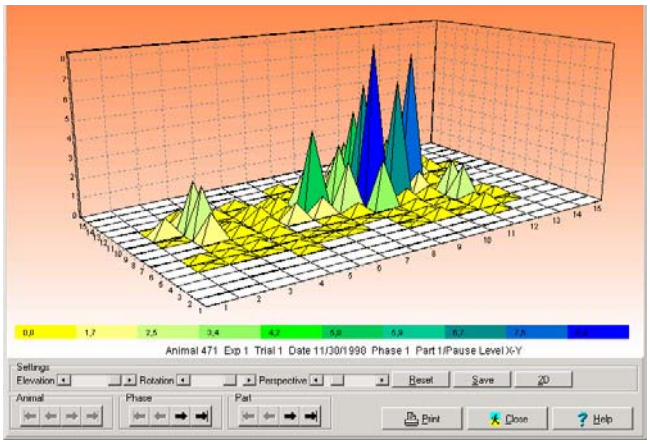
Column	Titel	Description
1	**Phase**	Phase number
2	**Part**	Part
3	**Dis**	Distance travelled (cm)
1st block		
4	**Rear**	Number of rearing events
5	**%**	Percent rearing time referred to total part duration
6	**XY**	Number of times the animal has been detected in the XY-level only
7	**%**	Percent XY time referred to total part duration
		The sum of Rear% and XY% is 100% (=total part duration)
8	**Gro**	Number of grooming events determined automatically using the threshold **Grooming**
9	**Man**	Frequency of manually entered freezing events
10	**Fre**	Number of freezing events determined automatically using the threshold **Freezing**
11	**FreD**	Total duration of all freezing events
12	**Jum**	Number of jumps
13	**Act**	Number of movement actions exceeding the **Activity** threshold
14	**%**	Percent activity time referred to total part duration
15	**Hyp**	Number of actions exceeding the **Hyperactivity** threshold
16	**%**	Percent hyperactivity time referred total part duration
2nd block		
17	**Vmean**	Mean speed (cm/s)
18	**Vmax**	Maximum speed (cm/s)
19	**VSD**	Standard deviation
20	**VSEM**	Standard error of arithmetic mean (SEM)
21	**Rest**	Resting time
22	**%**	Percent resting time referred to total part duration
23	**Expl%**	Percentage of box area that has been visited by the animal (**exploratory area**)

◆ ***The spacial analysis graph***

This graph provides an overview about the temporal and spacial distribution of the animal´s activity in the box using the coordinates detected in the XY-sensor level. For each phase and part a separate graph is generated.

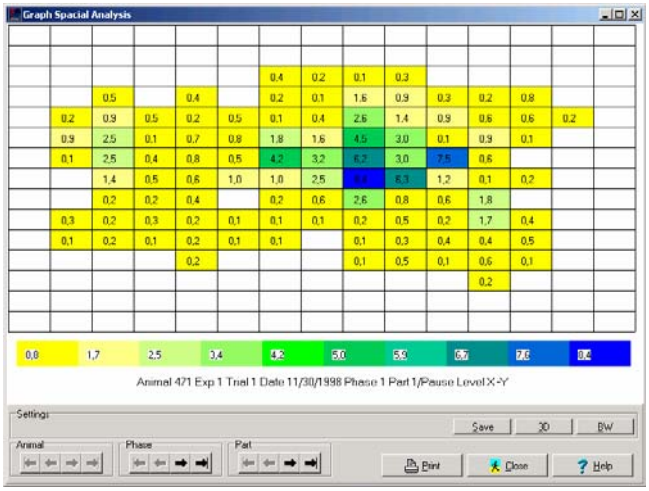
The total box area is divided into **N x N elements**. The maximum number of elements is dependant on the frame size. The number of elements can also be reduced by the user (averaging).

For each element the system now calculates the **total visit time** of the animal. The **percentage of visit time** for each element compared to the **total part time** is then output.



When the graph is called up data is shown in a **3-dimensional** coordinate system. The visit time can be read off from the height of the peaks and their color.

In the **2-dimensional display** the percentage visit time in each element is output as numerical value inside each element. In order to allow a quick overview about the animal´s length of stay the elements are shaded with a **pattern of dots** (black & white display) or with a specific **color** (color display).



The graph can be saved as is displayed as BMP-file.

◆ ***The spacial analysis table***

This table corresponds to the **spacial analysis graph**. It outputs the calculated percentages of visit time for each box element in form of a grid. The values are arranged in columns (x-coordinate) and lines (y-coordinate). The sum of all values is 100%.

1 Pause

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

◆ ***The behavior table***

This table gives information on the manually recorded behaviors for **Box 1** (marker keys 1...5) with their

⇒ frequency (**Qty.**),
⇒ total duration (**Dur.**) and
⇒ **latency** (time elapsed from start of part to first occurrence)

for each phase and part (“Type”).

Phase	Type	Key 1		Key 2		Key 3		Key 4		Key 5	
		Qty.	Dur.	Qty.	Dur.	Qty.	Dur.	Qty.	Dur.	Qty.	Dur.
1	Pause	2	0,6	2	0,6	2	0,7	0	0,0	0	0,0
	E-Stim	4	0,9	0	0,0	4	0,8	2	0,6	2	0,6
	Pause	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0
2	Pause	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0

Phase	Type	Key 1 Latency	Key 2 Latency	Key 3 Latency	Key 4 Latency	Key 5 Latency
1	Pause	14,3	19,4	25,3	0,0	0,0
	E-Stim	20,4	0,0	12,9	0,1	4,7
	Pause	0,0	0,0	0,0	0,0	0,0
2	Pause	0,0	0,0	0,0	0,0	0,0

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 all numerical tables in the form of special export files. Selectable column separators and decimal separators and the possibility of enclosing strings in quotation marks allow the adaptation of the file to the particular program used. The file is in the dBase-compatible CSV-format; this is supported by all professional statistics packages.

A special “Export in Wintrack Format” function generates a special file compatible with the **Wintrack** analysis program by David Wolfer, Zürich. Details about Wintrack can be found at

http://www.dpwolfer.ch/wintrack/Index.htm

Using the Fear Conditioning setup for other experiments

Light-Dark Test

The light dark test is a quick and easy-to-use test to evaluate the unconditioned anxiety level of the animal. It does not require prior training. 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 safe area, rodents spontaneously prefer the dark. Anxiolytic compounds increase 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.

In order to carry out anxiety tests special **light-dark boxes** with a central gate are available that are placed into the light-beam frame. The dark part covers either 33% or 50% of the total box area (*please specify on your order)*. These boxes do not have a floor so that the animal can either move on the grid floor or on one of the PVC plates provided with the acrylic FCS arena. The high-intensity lamps available in the FCS housing as an option can be used to increase aversiveness of the bright compartment (intensities of up to 700 Lux).

During the experiment the movement of the animal between the compartments is monitored.

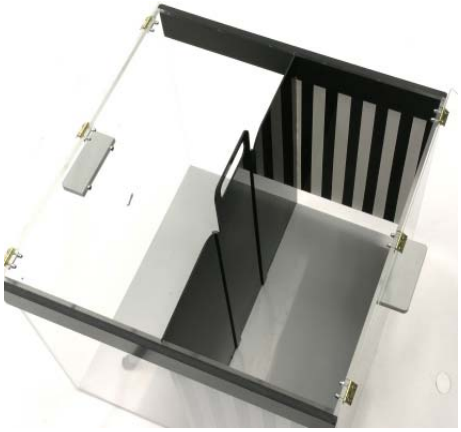
A special evaluation table is available in the Fear Conditioning software package that outputs for each compartment:

- the distance traveled,
- the time of stay,
- the number of visits (“transfers”),
- the latency first visit,
- the average speed and
- the number of rearings.

Part	Dis. [cm]	Time [s]	Trans.	Lat. [s]	Vmean [cm/s]	Rear
Comp 1						
Pause	1088.6	68.1	7	2.9	15.98	18
Comp 2						
Pause	1235.8	73.4	8	3.2	16.82	29

Place Preference Experiments

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.



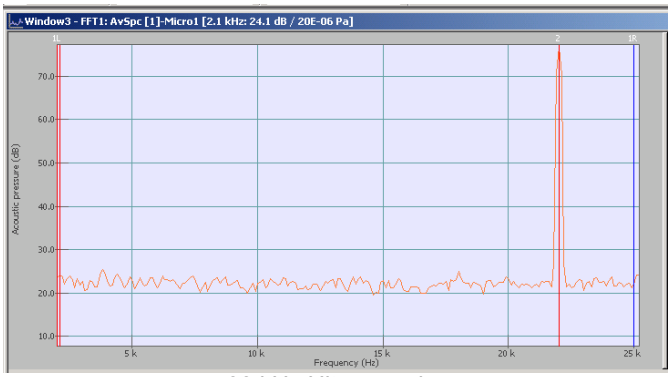
Special Place Preference arenas for rats & mice are available for the FCS setup. These 2-compartment arenas provide visual cues through differently patterened/colored walls and differently structured floor inserts to facilitate tactile discrimination. A manual door is used to restrict access of the animal to one compartment during conditioning. The high-resolution infrared sensors are used to monitor entries into compartments and locomotor activity.

The same parameters are calculated in this test as is described in the section “Light-Dark Test”.

Defensive Behaviors

The LARGE/ADVANCED system is now equipped with high-frequency loud-speakers as a standard that are able to generate ultrasound with frequencies up to **25kHz**. This new feature allows for the first time to use the FCS setup to evaluate defensive behaviors (“panic responses”) in rats.

It has been shown that playbacks of 22kHz ultrasonic vocalizations (“alarm calls”) of rats could induce defensive behaviors in other rats, i.e. escape followed by freezing (Blanchard et al. 1991, 1993). Similar to these natural sounds artificial ultrasound is able to elicit the behavior (Beckett et al. 1996). With the improved FCS setup this behavior can now be evaluated in detail and the effect of compounds on baseline, i.e. without sound stimulus, and on defensive behaviors, i.e. after 22kHz exposure, can be characterized. Escape behavior - defined as percentage of time spent in hyperactive locomotion – and % time freezing can be reliably detected - this opens a new dimension in drug screening.



22 kHz Ultrasound

The following recently published paper describes the use of the TSE FC system to study the sensitivity of the ultrasound induced defensive behaviors test to a variety of anxiolytics:

Nicolas LB, Klein S, Prinssen EP. Defensive-like behaviors induced by ultra-sound: further pharmacological characterization in Lister-hooded rats. Psychopharmacology 2007; 194: 243-52.

The TSE ActiMot system can also be used for controlling the Place Preference or the Light-Dark test (optional). The extension comprises a special control unit and a special interface that are connected to the FCS measuring unit instead of the standard FC components. In this configuration more than 100 different evaluation parameters allow a more complex analysis, among them: %distance traveled, time rearing, %time of stay, moving time, %moving time, resting time, %resting time... It is also possible to perform an interval analysis by selecting user-defined time intervals or to calculate a variety of parameters by taking either the complete arena space or user-defined zones into account.

This extension also converts the FCS setup into a comprehensive Open Field test system.

Please contact us if you are interested in this system extension.

Partial List of Users

- Bayerische Julius-Maximilians-Universität Würzburg, Würzburg, Germany
- Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany
- Consiglio Nazionale delle Ricerche - CNR, Roma, Italy
- Deutsches Krebsforschungszentrum - DKFZ, Heidelberg, Germany
- Eberhard Karls Universität Tübingen, Tübingen, Germany
- Ecole Polytechnique Federale de Lausanne - EPFL, Lausanne, Switzerland
- European Neuroscience Institute - ENI, Göttingen, Germany
- F. Hoffmann-La Roche AG, Basel, Switzerland
- GlaxoSmithKline R&D Ltd. - GSK, Harlow, Essex, Great Britain
- Harvard Medical School, Boston, MA, USA
- Ingenium Pharmaceuticals AG, Martinsried, Germany
- Instytut Psychiatrii i Neurologii - IPiN, Warsaw, Poland
- JSW-Research Forschungslabor GmbH, Graz, Austria
- King's College London, London, Great Britain
- Korean Adv. Inst. of Science & Technology - KAIST, Yusong-gu, Taejon, South Korea
- Ludwig-Maximilians-Universität München, Planegg-Martinsried, Germany
- Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Germany
- Max-Planck-Institut für Experimentelle Medizin, Göttingen, Germany
- Max-Planck-Institut für Medizinische Forschung, Heidelberg, Germany
- Medizinische Universität Innsbruck, Innsbruck, Austria
- Morgan State University, Baltimore, MD, USA
- Neurimmune Therapeutics AG, Zürich, Switzerland
- Northwestern University, Chicago, IL, USA
- NOVARTIS PHARMA AG, Basel, Switzerland
- Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany
- Polish Academy of Sciences, Krakow, Poland
- Semnan University of Medical Sciences, Semnan, Iran
- Technische Universität München, München, Germany
- University of Aarhus, Risskov, Denmark
- University of Aberdeen, Aberdeen, Great Britain
- University of California, Irvine, Irvine, CA, USA
- University of Copenhagen, Copenhagen, Denmark
- University of Helsinki, Helsinki, Finland
- University of Tartu, Tartu, Estland
- University of Toronto, Toronto, Ontario , Canada
- Universität Zürich, Zürich, Switzerland
- Vrije Universiteit Amsterdam, HV Amsterdam, The Netherlands
- Weizmann Institute of Science, Rehovot, Israel

- Westfälische Wilhelms-Universität Münster, Münster, Germany
- Zentralinstitut für Seelische Gesundheit, Mannheim, Germany

Publications

- **Abrari K, Rashidy-Pour A, Semnanian S, Fathollahi Y.** Administration of corticosterone after memory reactivation disrupts subsequent retrieval of a contextual conditioned fear memory: dependence upon training intensity. Neurobiology of Learning and Memory 2008; 89: 178-84.
- **Jamain S, Radyushkin K, Hammerschmidt K, Granon S, Boretius S, Varoqueaux F, Ramanantsoa N, Gallego J, Ronnenberg A, Winter D, Frahm J, Fischer J, Bourgeron T, Ehrenreich H, Brose N.** Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism. Proceedings of the National Academy of Sciences 2008; 105: 1710-5.
- **Lehner M, Taracha E, Turzyńska D, Sobolewska A, Hamed A, Kołomańska P, Skórzewska A, Maciejak P, Szyndler J, Bidziński A, Płaźnik A.** The role of the dorsomedial part of the prefrontal cortex serotonergic innervation in rat responses to the aversively conditioned context: Behavioral, biochemical and immunocytochemical studies. Behavioural Brain Research 2008; 192: 203-15.
- **Maciejak P, Lehner M, Turzynska D, Szyndler J, Bidzinski A, Taracha E, Sobolewska A, Walkowiak J, Skorzewska A, Wisłowska A, Hamed A, Plaznik A.** The opposite role of hippocampal mGluR1 in fear conditioning in kindled and non-kindled rats. Brain Research 2008; 1187: 184-93.
- **Wislowska-Stanek A, Hamed A, Lehner M, Bidzinski A, Turzynska D, Sobolewska A, Walkowiak J, Plaznik A.** Effects of midazolam and buspirone on in vivo concentration of amino acids and monoamine metabolites in the rat hippocampus. Pharmacological Reports 2008; 60: 209-18.
- **Fischer A, Radulovic M, Schrick C, Sananbenesi F, Godovac-Zimmermann J, Radulovic J.** Hippocampal Mek/Erk signaling mediates extinction of contextual freezing behavior. Neurobiology of Learning and Memory 2007; 87(1): 149-58.
- **Fischer A, Sananbenesi F, Wang X, Dobbin M, Tsai LH.** Recovery of learning and memory is associated with chromatin remodelling. Nature 2007; 447: 178-82.
- **Fullgrabe MW, Vengeliene V, Spanagel R.** Influence of age at drinking onset on the alcohol deprivation effect and stress-induced drinking in female rats. Pharmacology Biochemistry and Behavior 2007; 86: 320-6.
- **Nicolas LB, Klein S, Prinssen EP.** Defensive-like behaviors induced by ultra-sound: further pharmacological characterization in Lister-hooded rats. Psychopharmacology 2007; 194: 243-52.
- **Pugh PL, Vidgeon-Hart MP, Ashmeade T, Culbert AA, Seymour Z, Perren MJ, Joyce F, Bate ST, Babin A, Virley DJ, Richardson JC, Upton N, Sunter D**. Repeated administration of the noradrenergic neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) modulates neuroinflammation and amyloid plaque load in mice bearing amyloid precursor protein and presenilin-I mutant transgenes. Journal of Neuroinflammation 2007; 4: 8.
- **Sananbenesi F, Fischer A, Wang X, Schrick C, Neve R, Radulovic J, Tsai LH.** A hippocampal Cdk5 pathway regulates extinction of contextual fear. Nature Neuroscience 2007; 10(8): 1012-9.
- **Stiedl O, Misane I, Koch M, Pattij T, Meyer M, Ogren SO.** Activation of the brain 5-HT(2C) receptors causes hypolocomotion without anxiogenic-like cardiovascular adjustments in mice. Neuropharmacology 2007; 52(3): 949-57.
- **Todorovic C, Radulovic J, Jahn O, Radulovic M, Sherrin T, Hippel C, Spiess J.** Differential activation of CRF receptor subtypes removes stress-induced memory deficit and anxiety. European Journal of Neuroscience 2007; 25: 3385-97.
- **Zienowicz M, Wislowska-Stanek A, Lehner M, Taracha E, Skorzewska A, Bidzinski A, Turzynska D, Sobolewska A, Walkowiak J, Maciejak P, Szyndler J, Plaznik A**. Fluoxetine attenuates the effects of pentylenetetrazol on rat freezing behavior and c-Fos expression in the dorsomedial periaqueductal gray. Neuroscience Letters 2007; 414: 252-6.
- **Siren AL, Radyushkin K, Boretius S, Kammer D, Riechers CC, Natt O, Sargin D, Watanabe T, Sperling S, Michaelis T, Price J, Meyer B, Frahm J, Ehrenreich H.** Global brain atrophy after unilateral parietal lesion and its prevention by erythropoietin. Brain 2006; 129: 480-9.
- **Zueger M, Urani A, Chourbaji S, Zacher C, Lipp HP, Albrecht U, Spanagel R, Wolfer DP, Gass P.** mPer1 and mPer2 mutant mice show regular spatial and contextual learning in standardized tests for hippocampus-dependent learning. Journal of Neural Transmission 2006; 113(3): 347-56.
- **Von Bohlen und Halbach O, Zacher C, Gass P, Unsicker K.** Age-related alterations in hippocampal spines and deficiencies in spatial memory in mice. Journal of Neuroscience Research 2006; 83: 525-31.
- **Fischer A, Sananbenesi F, Pang PT, Tsai L.** Opposing roles of transient and prolonged expression of p25 in synaptic plasticity and hippocampus-dependent memory. Neuron 2005; 48: 825-38.

- **Misane I, Tovote P, Meyer M, Spiess J, Ogren SO, Stiedl O.** Time-dependent involvement of the dorsal hippocampus in trace fear conditioning in mice. Hippocampus 2005; 15(4): 418-26.
- **Radyushkin K, Anokhin K, Meyer BI, Jiang Q, Alvarez-Bolado G, Gruss P.** Genetic ablation of the mammillary bodies in the Foxb1 mutant mouse leads to selective deficit of spatial working memory. European Journal of Neuroscience 2005; 21: 219-29.
- **Raud S, Innos J, Abramov U, Reimets A, Kõks S, Soosaar A, Matsui T, Vasar E.** Targeted invalidation of CCK2 receptor gene induces anxiolytic-like action in light-dark exploration, but not in fear conditioning test. Psychopharmacology 2005; 181: 347-57.
- **Ridder S, Chourbaji S, Hellweg R, Urani A, Zacher C, Schmid W, Zink M, Hortnagl H, Flor H, Henn FA, Schutz G, Gass P.** Mice with genetically altered glucocorticoid receptor expression show altered sensitivity for stress-induced depressive reactions. The Journal of Neuroscience 2005; 25(26): 6243-50.
- **Stiedl O, Meyer M, Jahn O, Ogren SO, Spiess J.** Corticotropin-releasing factor receptor 1 and central heart rate regulation in mice during expression of conditioned fear. The Journal of Pharmacology and Experimental Therapeutics 2005; 312: 905-16.
- **Tornberg J, Voikar V, Savilahti H, Rauvala H, Airaksinen MS.** Behavioural phenotypes of hypomorphic KCC2-deficient mice. European Journal of Neuroscience 2005; 21: 1327-37.
- **Tovote P, Meyer M, Pilz PKD, Ronnenberg A, Ogren SO, Spiess J, Stiedl O.** Dissociation of temporal dynamics of heart rate and blood pressure responses elicited by conditioned fear but not acoustic startle. Behavioral Neuroscience 2005; 119(1): 55-65.
- **Tovote P, Meyer M, Ronnenberg A, Ogren SO, Spiess J, Stiedl O.** Heart rate dynamics and behavioral responses during acute emotional challenge in corticotropin-releasing factor receptor 1-deficient and corticotropin-releasing factor-overexpressing mice. Neuroscience 2005; 134(4): 1113-22.
- **Voikar V, Polus A, Vasar E, Rauvala H.** Long-term individual housing in C57BL/6J and DBA/2 mice: assessment of behavioral consequences. Genes, Brain and Behavior 2005; 4: 240-52.
- **Wislowska-Stanek A, Zienowicz M, Lehner M, Taracha E, Bidzinski A, Maciejak P, Skórzewska A, Szyndler J, Plaznik A.** Buspirone attenuates conditioned fear-induced c-Fos expression in the rat hippocampus. Neuroscience Letters 2005; 389: 115-20.
- **Ahi J, Radulovic J, Spiess J.** The role of hippocampal signaling cascades in consolidation of fear memory. Behavioural Brain Research 2004; 149: 17-31.
- **Fischer A, Sananbenesi F, Schrick C, Spiess J, Radulovic J.** Distinct roles of hippocampal de novo protein synthesis and actin rearrangement in extinction of contextual fear. The Journal of Neuroscience 2004; 24(8): 1962-6.
- **Stiedl O, Tovote P, Ogren SO, Meyer M.** Behavioral and autonomic dynamics during contextual fear conditioning in mice. Autonomic Neuroscience: Basic and Clinical 2004; 115(1-2): 15-27.
- **Stork O, Zhdanov AV, Kudersky A, Yoshikawa T, Obata K, Pape HC.** Neuronal functions of the novel serine / threonine kinase Ndr2. The Journal of Biological Chemistry 2004; 279(44): 45773-81.
- **Tovote P, Meyer M, Beck-Sickinger AG, Von Horsten S, Ogren SO, Spiess J, Stiedl O.** Central NPY receptor-mediated alteration of heart rate dynamics in mice during expression of fear conditioned to an auditory cue. Regulatory Peptides 2004; 120: 205-14.
- **Voikar V, Rossi J, Rauvala H, Airaksinen MS.** Impaired behavioural flexibility and memory in mice lacking GDNF family receptor alpha2. European Journal of Neuroscience 2004; 20(1): 308-12.
- **Voikar V, Vasar E, Rauvala H.** Behavioral alterations induced by repeated testing in C57BL/6J and 129S2/Sv mice: implications for phenotyping screens. Genes, Brain and Behavior 2004; 3: 27-38.
- **Balschun D, Wolfer DP, Gass P, Mantamadiotis T, Welzl H, Schuetz G, Frey JU, Lipp HP.** Does cAMP response element-binding protein have a pivotal role in hippocampal synaptic plasticity and hippocampus-dependent memory. The Journal of Neuroscience 2003; 23(15): 6304-14.
- **Blank T, Nijholt I, Grammatopoulos DK, Randeva HS, Hillhouse EW, Spiess J.** Corticotropin-releasing factor receptors couple to multiple G-proteins to activate diverse intracellular signaling pathways in mouse hippocampus: role in neuronal excitability and associative learning. The Journal of Neuroscience 2003; 23(2): 700-7.
- **Blank T, Nijholt I, Kye MJ, Radulovic J, Spiess J.** Small-conductance, Ca2+-activated K+ channel SK3 generates age-related memory and LTP deficits. Nature Neuroscience 2003; 6(9): 911-2.
- **Fischer A, Sananbenesi, Schrick C, Spiess J, Radulovic J.** Regulation of contextual fear conditioning by baseline and inducible septo-hippocampal cyclin-dependent kinase 5. Neuropharmacology 2003; 44: 1089-99.
- **Fleischmann A, Hvalby O, Jensen V, Strekalova T, Zacher C, Layer LE, Kvello A, Reschke M, Spanagel R, Sprengel R, Wagner EF, Gass P.** Impaired long-term memory and NR2A-type NMDA receptor-dependent synaptic plasticity in mice lacking c-FOS in the CNS. The Journal of Neuroscience 2003; 23(27): 9116-22.

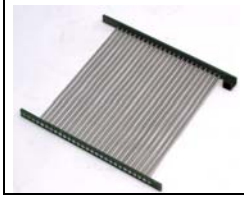
- **Laxmi TR, Stork O, Pape HC.** Generalisation of conditioned fear and its behavioral expression in mice. Behavioural Brain Research 2003; 145: 89-98.
- **Maciejak P, Taracha E, Lehner M, Szyndler J, Bidzinski A, Skorzewska A, Wislowska A, Zienowicz M, Plaznik A.** Hippocampal mGluR1 and consolidation of contextual fear conditioning. Brain Research Bulletin 2003; 62: 39-45.
- **Sananbenesi F, Fischer A, Schrick C, Spiess J, Radulovic J.** Mitogen-activated protein kinase signaling in the hippocampus and its modulation by corticotropin-releasing factor receptor 2: a possible link between stress and fear memory. The Journal of Neuroscience 2003; 23(36): 11436-43.
- **Seidenbecher T, Laxmi TR, Stork O, Pape HC.** Amygdalar and hippocampal theta rhythm synchronization during fear memory retrieval. Science 2003; 301(5634): 846-50.
- **Stiedl O, Meyer M, Kishimoto T, Rosenfeld MG, Spiess J.** Stress-mediated heart rate dynamics after deletion of the gene encoding corticotropin-releasing factor receptor 2. European Journal of Neuroscience 2003; 17: 2231-5.
- **Strekalova T, Zoerner B, Zacher C, Sadovska G, Herdegen T, Gass P.** Memory retrieval after contextual fear conditioning induces c-Fos and JunB expression in CA1 hippocampus. Genes, Brain and Behavior 2003; 2(1): 3-10.
- **Blank T, Nijholt I, Eckart K, Spiess J.** Priming of long-term potentiation in mouse hippocampus by corticotropin-releasing factor and acute stress: implications for hippocampus-dependent learning. The Journal of Neuroscience 2002; 22(9): 3788-94.
- **Fischer A, Sananbenesi F, Schrick C, Spiess J, Radulovic J.** Cyclin-dependent kinase 5 is required for associative learning. The Journal of Neuroscience 2002; 22(9): 3700-07.
- **Sananbenesi F, Fischer A, Schrick C, Spiess J, Radulovic J.** Phosphorylation of hippocampal Erk-1/2, Elk-1, and p90-Rsk-1 during contextual fear conditioning: interactions between Erk-1/2 and Elk-1. Molecular And Cellular Neuroscience 2002; 21: 463-76.
- **Lee HJ, Berger SY, Stiedl O, Spiess J, Kim JJ.** Post-training injections of catecholaminergic drugs do not modulate fear conditioning in rats and mice. Neuroscience Letters 2001; 303: 123-26.
- **Otto C, Kovalchuk Y, Wolfer DP, Gass P, Martin M, Zuschratter W, Grone HJ, Kellendonk C, Tronche F, Maldonado R, Lipp HP, Konnerth A, Schuetz G**. Impairment of mossy fiber long-term potentiation and associative learning in pituitary adenylate cyclase activating polypeptide type I receptor-deficient mice. The Journal of Neuroscience 2001; 21: 5520-7.
- **Stork O, Stork S, Pape HC, Obata K.** Identification of genes expressed in the amygdala during the formation of fear memory. Learning & Memory 2001; 8: 209-19.
- **Kishimoto T, Radulovic J, Radulovic M, Lin C, Schrick C, Hooshmand F, Hermanson O, Rosenfeld M, Spiess J.** Deletion of Crhr2 reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. Nature Genetics 2000; 24: 415-9.
- **Stiedl O, Birkenfeld K, Palve M, Spiess J.** Impairment of conditioned contextual fear of C57BL/6J mice by intracerebral injections of the NMDA receptor antagonist APV. Behavioural Brain Research 2000; 116: 157-68.
- **Stiedl O, Misane I, Spiess J, Ogren SO.** Involvement of the 5-HT1A receptors in classical fear conditioning in C57BL/6J mice. The Journal of Neuroscience 2000; 20(22): 8515-27.
- **Radulovic J, Ruhmann A, Liepold T, Spiess J.** Modulation of learning and anxiety by corticotropin-relasing factor (CRF) and stress: differential roles of CRF receptors 1 and 2. The Journal of Neuroscience 1999; 19(12): 5016-25.
- **Stiedl O, Palve M, Radulovic J, Birkenfeld K, Spiess J.** Differential impairment of auditory and contextual fear conditioning by protein synthesis inhibition in C57BL/6N Mice. Behavioral Neuroscience 1999; 113(3): 496-506.
- **Stiedl O, Radulovic J, Lohmann R, Birkenfeld K, Palve M , Sananbenesi F, Spiess J.** Strain and substrain differences in context-and tone-dependent fear conditioning of inbred mice. Behavioural Brain Research 1999; 104: 1-12.
- **Gass P, Wolfer DP, Balschun D, Rudolph D, Frey U, Lipp HP, Schuetz G.** Deficits in memory tasks of mice with CREB mutations depend on gene dosage. Learning & Memory 1998; 5: 274-88.
- **Milanovic S, Radulovic J, Laban O, Stiedl O, Henn F, Spiess J.** Production of the Fos protein after contextual fear conditioning of C57BL/6N mice. Brain Research 1998; 784: 37-47.
- **Radulovic J, Kammermeier J, Spiess J.** Relationship between Fos production and classical fear conditioning: effects of novelty, latent inhibition, and unconditioned stimulus preexposure. The Journal of Neuroscience 1998; 18(18): 7452-61.
- **Radulovic J, Kammermeier J, Spiess J.** Generalization of fear responses in C57BL/6N mice subjected to one-trial foreground contextual fear conditioning. Behavioural Brain Research 1998; 95: 179-89.
- **Stiedl O, Spiess J.** Effect of tone-dependent fear conditioning on heart rate and behavior of C57BL/6N mice. Behavioral Neuroscience 1997; 111(4): 703-11.

Ordering Information

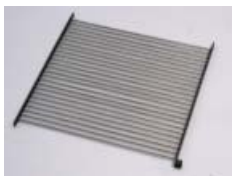
A complete system consists of:

1. **N** x Measuring Units ADVANCED, LARGE or SMALL
2. 1 Control Unit suited for **N** units
3. Software Package for **N** measuring units
4. Your choice of options and accessories

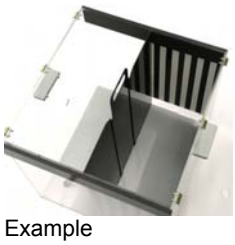
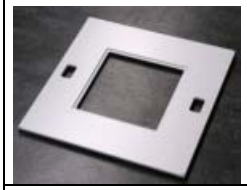
N= Number of measuring units (1, 2 or 4)*

Cat.No.	Description
ADVANCED SMALL	***For mouse only***
1. Measuring Unit	***Complete unit with housing, frame, arena & foot shock grid***
303411-ADV-SMALL-MEAS-UNIT-8-G   	**Fear Conditioning Measuring Unit ADVANCED/SMALL** Comprising: 303411-ADV-SMALL-HOUSING-8 Housing Sound-attenuating housing with loudspeaker (background noise, stimulus sound) and 2 symmetrically mounted lamps (continuous house light; right and left lamps can be used independently) in ceiling construction, ventilation fan in side wall, sliding floor and cable outlet. Door with hinges on one side and observation window that can be closed. Central hole in ceiling construction (60mm diameter) for camera observation with optional video kit. Dimensions: 520x520x650 mm (WxDxH) 303411-ADV-SMALL-BASE Base Frame Base construction, outer size approx. 310x310 mm, with stainless steel excrement tray, with black finish to remove reflections. Support for removable foot shock grid. Equipped with infrared sensors for detection of animal location in 2 levels (XY-level for horizontal, Z-level for vertical movement). 16 sensor pairs each on X-, Y- and Z-axis with sensor distance 14mm (high-resolution). XY- and Z-level are steplessly adjustable. Incl. 2 pcs. of height adjustment tools for Z-level. 303411-ADV-SMALL-ARENA-CL Arena TRANSPARENT Standard conditioning arena made from clear acrylic, without floor. Removable lid made from clear acrylic with circular cut-out (150 mm diameter). Dimensions: 230x230x350 mm (WxDxH). With 2 PVC grid cover plates (gray and black) used to modify the context. 303411-ADV-SMALL-GRID-8 Foot Shock Grid Grid rod floor made from stainless steel, with 4 mm rod diameter, 8.9 mm rod distance (center to center). With connector for shock cable. **Requires:** o 303411-CU-**N**-8 Fear Conditioning Control Unit o 303411-SW-**N** Fear Conditioning Software Package **Optional:** o 303411-LED External LED unit used during manual freezing observation o 303411-STIM-LIGHT Additional extra-bright stimulus light o 303411-PEEP-HOLE Peep hole **N= Number of measuring units (1, 2 or 4)***
2. Foot Shock Grid	
303411-ADV-SMALL-GRID-8 	**Foot shock grid** Grid rod floor made from stainless steel, with 4 mm rod diameter, 8.9 mm rod distance (center to center). With connector for shock cable. *Please note that the foot shock grid is already included in the measuring unit 303411-ADV-SMALL-MEAS-UNIT-8-G*

3. Arenas	
303411-ADV-SMALL-ARENA-CL 	**Arena TRANSPARENT** Standard conditioning arena made from clear acrylic, without floor. Removable lid made from clear acrylic with circular cut-out (150 mm diameter). Dimensions: 230x230x350 mm (WxDxH). With 2 PVC grid cover plates (gray and black) used to modify the context. **Requires**: o 303411-ADV-SMALL-GRID-X Foot shock grid *Please note that the transparent arena is already included in the measuring unit 303411-ADV-SMALL-MEAS-UNIT-8-G*
303411-ADV-SMALL-ARENA-IR 	**Arena BLACK** Used to modify the context. Made from black acrylic (permeable to infrared light), without floor. Removable lid made from black acrylic with circular cut-out (150mm diameter). Dimensions: 230x230x350 mm (WxDxH). With 2 PVC grid cover plates (gray and black). **Requires**: o 303411-ADV-SMALL-GRID-X Foot shock grid
303411-ADV-SMALL-ARENA-LD-33 	**Light/Dark Box 33%** Arena with one bright and one dark compartment, made of clear and black acrylic (permeable to infrared light), without floor. The black compartment covers 33% of the arena area. The black dividing wall (not removable; permeable to infrared light) is equipped with a central tunnel gate (50x55 mm (WxH)). With 2 lids (clear and black). Dimensions: 230x230x350 mm (WxDxH). The arena is placed on the grid; the grid cover plates provided with the clear arenas can also be used together with this arena. **Requires:** o 303411-ADV-SMALL-GRID-X Foot shock grid
303411-ADV-SMALL-ARENA-LD-50	**Light/Dark Box 50%** Arena with one bright and one dark compartment, made of clear and black acrylic (permeable to infrared light), without floor. The black compartment covers 50% of the arena area. The black dividing wall (not removable; permeable to infrared light) is equipped with a central tunnel gate (50x55 mm (WxH)). With 2 lids (clear and black). Dimensions: 230x230x350 mm (WxDxH). The arena is placed on the grid; the grid cover plates provided with the clear arenas can also be used together with this arena. **Requires**: o 303411-ADV-SMALL-GRID-X Foot shock grid
303411-ADV-LD-DOOR	**Option Sliding Door (Manually Operated)** for Light/Dark Box SMALL The dividing wall of the light/dark box is equipped with a black sliding door. In order to operate the door the housing doors have to be open. Has to be ordered together with the Light-/Dark-Box SMALL.
303411-ADV-SMALL-ARENA-PP	**Place Preference Arena** This 2-compartment arena is made of transparent acrylic, without floor. The removable lid is made of transparent acrylic. The arena is divided into two equally sized compartments by a separating wall of black acrylic plastic (permeable to infrared light). This divider is equipped with a central gate (50x55 mm (WxH)). This gate can be opened and closed by a manually operated sliding door made of black acrylic plastic. The door is used to restrict access to only one compartment during the conditioning phase. Dimensions: 230x230x350 mm (WxDxH). The two compartments differ in their wall design (visual discrimination). Compartment

	1: 2 opposing walls with a vertical pattern of black/white stripes, compartment 2: transparent acrylic plastic or with a black or white coating (please specify with your order). The right and left sides of both compartments are made of transparent acrylic plastic. Customer-specific wall arrangements are possible. A special floor insert set is available for tactile discrimination. The set includes 5 different bases, each with the size of one compartment. The inserts are placed on the grid and can be combined with each other at will. The standard package comprises: 3 different perforated plates as well as 2 light gray PVC plates with coarse and smoothly structured surfaces. If only one base insert is used then the standard floor grid can also be used for the floor of the other compartment. Customer-specific base inserts can also be manufactured. **Requires**: o 303411-ADV-SMALL-GRID-X Foot shock grid
ADVANCED LARGE	***For rat and/or mouse***
1. Measuring Unit	***The ADVANCED LARGE measuring unit has to be completed with your choice of grid (rat or mouse)***
303411-ADV-LARGE-MEAS-UNIT-8-NG   	**Fear Conditioning Measuring Unit ADVANCED/LARGE** Comprising: 303411-ADV-LARGE-HOUSING-8 Housing Sound-attenuating housing with loudspeaker (background noise, stimulus sound) and 4 symmetrically mounted lamps (continuous house light; right and left lamps can be used independently) in ceiling construction, ventilation fan in side wall, sliding floor and cable outlet. Double door with hinges on both sides and peep hole in the door on the right. Central hole in ceiling construction (60mm diameter) for camera observation with optional video kit. Dimensions: 730x730x830 mm (WxDxH) 303411-ADV-LARGE-BASE Base Frame Base construction, outer size approx. 540x540 mm, with stainless steel excrement tray, with black finish to remove reflections. Support for removable foot shock grid. Equipped with infrared sensors for detection of animal location in 2 levels (XY-level for horizontal, Z-level for vertical movement). 32 sensor pairs each on X-, Y- and Z-axis with sensor distance 14 mm (high-resolution). XY- and Z-level are steplessly adjustable. Incl. 2 pcs. of height adjustment tools for Z-level. 303411-ADV-LARGE-ARENA-LARGE-CL Arena TRANSPARENT LARGE Standard conditioning arena for rats. Can also be used for mice whenever a larger exploratory area is required. Clear acrylic, without floor. With removable lid (clear acrylic) with circular cut-out (290 mm diameter). Dimensions: 460x460x470 mm (WxDxH). With 2 PVC grid cover plates (gray and black) used to modify the context **Requires**: o 303411-ADV-LARGE-GRID-R-8 Foot Shock Grid Rat or 303411-ADV-LARGE-GRID-M-8 Foot Shock Grid Mouse o 303411-CU-**N**-8 Fear Conditioning Control Unit o 303411-SW-**N** Fear Conditioning Software Package **Optional**: o 303411-LED External LED unit used during manual freezing observation o 303411-STIM-LIGHT Additional extra-bright stimulus light **N= Number of measuring units (1, 2 or 4)***
2. Foot Shock Grid	
303411-ADV-LARGE-GRID-R-8 	**Foot shock grid RAT** Grid rod floor made from stainless steel, with 6 mm rod diameter, 19.5 mm rod distance (center to center). With connector for shock cable.
303411-ADV-LARGE-GRID-M-8	**Foot shock grid MOUSE** Grid rod floor made from stainless steel, with 4 mm rod diameter, 8.9 mm rod distance (center to center). With connector for shock cable. Suitable for use with large or small arenas.

	
3. Arenas	
Large Arenas & Accessories	
303411-ADV-LARGE-ARENA-LARGE-CL 	**Arena TRANSPARENT LARGE** Standard conditioning arena for rats. Can also be used for mice whenever a larger exploratory area is required. Clear acrylic, without floor. With removable lid made from clear acrylic with circular cut-out (290 mm diameter). Dimensions: 460x460x470 mm (WxDxH). With 2 PVC grid cover plates (gray and black) used to modify the context. **Requires**: o 303411-ADV-LARGE-GRID-X Foot shock grid *Please note that this arena is already included in the 303411-ADV-LARGE-MEAS-UNIT-8-NG Measuring Unit ADVANCED LARGE*
303411-ADV-LARGE-ARENA-L-IR 	**Arena BLACK LARGE** Used to modify the context. Suitable for rats and mice. Side walls made from black acrylic (permeable to infrared light). The removable black acrylic lid is provided with a circular cut-out (290 mm diameter). With fixed floor made from black PVC and additional floor cover plate made from PVC gray. Dimensions: 460x460x400 mm (WxDxH).
303411-ADV-LARGE-ARENA-LD-L-33 	**Light/Dark Box 33% LARGE** Large arena for rats and mice. 2 compartments made of clear and black acrylic (permeable to infrared light), without floor. The black compartment covers 33% of the arena area. With 2 lids (clear and black), 1 for each compartment. Dimensions: 450x450x450 mm (WxDxH). The arena is placed on the grid; alternatively the grid cover plates provided with the clear arenas can be used together with this arena. Without divider (has to be ordered separately). **Requires**: o 303411-ADV-LARGE-DIVIDER-LD-L-M Divider Mouse or 303411-ADV-LARGE-DIVIDER-LD-L-R Divider Rat o 303411-ADV-LARGE-GRID-X Foot shock grid
303411-ADV-LARGE-ARENA-LD-L-50	**Light/Dark Box 50% LARGE** Large arena for rats and mice. 2 compartments made of clear and black acrylic (permeable to infrared light), without floor. The black compartment covers 50% of the arena area. With 2 lids (clear and black), 1 for each compartment. Dimensions: 450x450x450 mm (WxDxH). The arena is placed on the grid; alternatively the grid cover plates provided with the clear arenas can be used together with this arena. Without divider (has to be ordered separately). **Requires**: o 303411-ADV-LARGE-DIVIDER-LD-L-M Divider Mouse or 303411-ADV-LARGE-DIVIDER-LD-L-R Divider Rat o 303411-ADV-LARGE-GRID-X Foot shock grid
303411-ADV-LARGE-DIVIDER-LD-L-M 	**Option Divider Mouse** for Light/Dark Box LARGE Black removable dividing wall permeable to infrared light equipped with a central tunnel gate (50x55 mm WxH). **Requires**: o 303411-ADV-LARGE-ARENA-LD-L-33 or 303411-ADV-LARGE-ARENA-LD-L-50 Light/Dark-Box LARGE **Optional**: o 303411-ADV-LD-DOOR Sliding Door

303411-ADV-LARGE-DIVIDER-LD-L-R 	**Option Divider Rat** for Light/Dark Box LARGE Black removable dividing wall permeable to infrared light equipped with a central tunnel gate (106x104 mm WxH). **Requires**: o 303411-ADV-LARGE-ARENA-LD-L-33 or 303411-ADV-LARGE-ARENA-LD-L-50 Light/Dark-Box LARGE **Optional**: o 303411-ADV-LD-DOOR Sliding Door
303411-ADV-LD-DOOR 	**Option Sliding Door (Manually Operated)** for Light/Dark Box LARGE The dividing wall of the light/dark box is equipped with a black sliding door. In order to operate the door the housing doors have to be open. Has to be ordered together with the Light-/Dark-Box LARGE with dividing wall.
303411-ADV-LARGE-ARENA-PP  Example	**Place Preference Arena** This 1-size 2-compartment arena for rats & mice is made of transparent acrylic, without floor. Dimensions: 460x460x400 mm (WxDxH). The removable lid is made of transparent acrylic. The arena is divided into two equally sized compartments by a removable separating wall of black acrylic plastic (permeable to infrared light). This divider is equipped with a central gate. 2 dividers, one with a large gate for rats rat gate and one with mouse gate are provided (rat: 106x104 mm, mouse: 50x55 mm (WxH)). This gate can be opened and closed by a manually operated sliding door made of black acrylic plastic. The door is used to restrict access to only one compartment during the conditioning phase. The compartments differ in their wall design (visual discrimination). Compartment 1: 2 opposing walls with a vertical pattern of black/white stripes, compartment 2: transparent acrylic plastic or with a black or white coating (please specify with your order). The right and left sides of both compartments are made of transparent acrylic plastic. Customer-specific wall arrangements are possible. A special floor insert set is available for tactile discrimination. The set includes 5 different bases, each with the size of one compartment. The inserts are placed on the grid and can be combined with each other at will. The standard package comprises: 3 different perforated plates as well as 2 light gray PVC plates with coarse and smoothly structured surfaces. If only one base insert is used then the standard floor grid can also be used for the floor of the other compartment. Customer-specific base inserts can also be manufactured. **Requires**: o 303411-ADV-LARGE-GRID-X Foot shock grid
Small Arenas & Accessories	
303411-ADV-LARGE-TEMPLATE 	**Template** Required for use of small arenas in the Fear Conditioning Measuring Unit ADVANCED/LARGE.
303411-ADV-LARGE-ARENA-SMALL-CL	**Arena TRANSPARENT SMALL** Conditioning arena for mice, whenever a smaller exploratory area is sufficient. Clear acrylic, without floor; the animal has access to the foot shock grid. With removable lid (clear acrylic) with circular cut-out (150 mm diameter). Dimensions: 230x230x350 mm (WxDxH). The grid cover plates provided with the large arenas can be used together with this small arena.

	**Requires**: o 303411-ADV-LARGE-TEMPLATE Template
303411-ADV-LARGE-ARENA-SMALL-IR 	**Arena BLACK SMALL** Used to modify the context. For mice, suited whenever a smaller exploratory area is sufficient. Side walls made from black acrylic (permeable to infrared light), without floor; the animal has access to the foot shock grid. Removable acrylic lid with circular cut-out (150 mm diameter). Dimensions: 230x230x350 mm (WxDxH). The grid cover plates provided with the large arenas can be used together with this small arena. **Requires**: o 303411-ADV-LARGE-TEMPLATE Template
303411-ADV-LARGE-ARENA-LD-S-33 	**Light/Dark Box 33% SMALL** Small arena for mice. 2 compartments made of clear and black acrylic (permeable to infrared light), without floor. The black compartment covers 50% of the arena area. The black dividing wall (not removable; permeable to infrared light) is equipped with a central tunnel gate (50x55 mm (WxH)). With 2 lids (clear and black), 1 for each compartment. Dimensions: 230x230x350 mm (WxDxH). The arena is placed into the template so that the grid is used as floor. The grid cover plates provided with the large arenas can be used together with this small arena. **Optional**: o 303411-ADV-LD-DOOR Sliding Door **Requires**: o 303411-ADV-LARGE-TEMPLATE Template
303411-ADV-LARGE-ARENA-LD-S-50	**Light/Dark Box 50% SMALL** Small arena for mice. 2 compartments made of clear and black acrylic (permeable to infrared light), without floor. The black compartment covers 50% of the arena area. The black dividing wall (not removable; permeable to infrared light) is equipped with a central tunnel gate (50x55 mm (WxH)). With 2 lids (clear and black), 1 for each compartment. Dimensions: 230x230x350 mm (WxDxH). The arena is placed into the template so that the grid is used as floor. The grid cover plates provided with the large arenas can be used together with this small arena. **Optional**: o 303411-ADV-LD-DOOR Sliding Door **Requires**: o 303411-ADV-LARGE-TEMPLATE Template
303411-ADV-LD-DOOR	**Option Sliding Door (Manually Operated)** for Light/Dark Box SMALL The dividing wall of the light/dark box is equipped with a black sliding door. In order to operate the door the housing doors have to be open. Has to be ordered together with the Light-/Dark-Box SMALL.
Options I	***These options have to be ordered together with the system. Retrofitting of existing systems is not possible***
1. Measuring Unit ADVANCED/SMALL	
303411-PEEP-HOLE	**Option Peep Hole** for Fear Conditioning Housing ADVANCED/SMALL The peep hole is mounted in the housing door in addition to the observation window

	and allows an overview of the whole arena without the animal being disturbed by incidence of light. Can only be used if an arena made of transparent material is used.
2. All Measuring Units	
303411-STIM-LIGHT  	**Option High-Intensity Stimulus Light** for all Fear Conditioning Housings 2 high-intensity LEDs are mounted symmetrically in the ceiling in addition to the house light that allow the generation of high-intensity light signals (up to 700 lux at the base of the arena). They are used in the Fear Conditioning experiment as software-controlled light stimulus in parallel or instead of sound stimulus. A switch on the control unit allows the left-hand and right-hand lamp to be switched independently from each other. The house light is operated manually if stimulus LEDs are present (toggle switch mounted on the housing side). If used together with a Light-Dark arena these lamps allow the degree of aversity of the bright compartment to be varied in accordance with the requirements of the experiment.
303411-HOUSING-DARK	**Option Housing Color Dark** for all Fear Conditioning Housings The housing is made from dark gray material (standard = white). This reduces the aversity of the interior. In a system with several measuring places differently colored housings can be used for Training and Retention tests. The dark color then serves as an altered context.
Options II	***These options can also be ordered separately***
303411-LED 	**External Signal Light "LED Unit"** for all Fear Conditioning Measuring Units This small signal unit can be attached to each FCS housing. The built-in LED is switched on by the software at user-defined intervals and for a user-defined duration. It supports the user in the manual recording of freezing events by indicating the time at which the condition of the animal (freezing or not) is to be evaluated by the observer and entered in the system via the keyboard. Cable length: 2m (other lengths on request).
303410-VID-KIT-2   	**Video-Kit** For Fear Conditioning System with DVD Recorder (with hard disk 200 GB)** Comprising: o Black & white CCD-camera with lens o Camera mounting device o DVD recorder with hard disk 200GB o Video-VGA-converter o Cable set For one FCS measuring unit. The FCS control unit triggers recording automatically when the experiment is started so that the recorded video and the FCS data file are synchronized. **Requires**: o 1 monitor (recommendation: 15" or 17" TFT). When using several FCS measuring units & video-kits, we recommend the Split Screen Adapter 303410-VID-KIT/SPSC-4 for simultaneous monitoring of up to 4 cameras on one screen (split screen) and a 17" TFT monitor. ***The video-kit is also available with DVD recorder without hard disk and videorecorder. Please contact us.*
303410-VID-KIT/SPSC-4	**Split Screen Adapter** for Fear Conditioning System

	For simultaneous monitoring of up to 4 cameras on one screen - split screen technology, when using multiple FCS Measuring Units & Video Kits. Recommendation: 17" TFT monitor.
Control Unit 	All control units comprise: - Sound generator: sinus 2-20kHz for FCS Measuring Unit ADVANCED/SMALL resp. 2-25kHz for FCS Measuring Unit ADVANCED/LARGE, adjustable in steps of 100Hz, with integrated amplifier (max. 90 dB) - Noise generator (white noise) with integrated amplifier (max. 100 dB) - Power supply for house light: max. 200 Lux for FCS Measuring Unit ADVANCED/SMALL resp. 100 Lux for FCS Measuring Unit ADVANCED/LARGE (measured in center of arena) - Shocker module (max 3.1 mA, adjustable in steps of 0.1mA). Sound frequency and amplitude, noise amplitude and brightness are manually adjusted on the control unit front panel. Left and right side of the housing illumination can be switched on and off independently from each other. The integrated shocker module with current flow detector and all electronics needed for automatically switching polarity of grid rods is suitable for very short shock events (min. 1 sec). The shock module is designed to provide constant current stimulation with software-adjustable output in user-defined steps of 0.1mA (up to 1.55mA maximum amplitude in steps of 0.05mA **optional**). Alternatively pulsating current with 20 Hz frequency can be applied (other frequencies on request). The current is applied independent from the actual cross-over resistance (max. 66 kOhm at 1.5 mA). Including TTL output (BNC jack) for control of external devices such as the TSE Video-Kit or custom data acquisition systems. PCI interface for PC. Upgradable for additional measuring units. **Optional:** o 303411-CU-STIM-NOISE Stimulus Noise o 303411-CU-SHOCK-1.55 Schock Amplitude 1.55mA o 303411-CU-TRIGGER Trigger Package
303411-CU-1-8	**Fear Conditioning Control Unit 1-Place** for control of 1 FCS Measuring Unit **Requires:** o 303411-SW-1 Fear Conditioning Software Package 1-Place
303411-CU-2-8	**Fear Conditioning Control Unit 2-Place** for control of up to 2 FCS Measuring Units **Requires**: o 303411-SW-2 Fear Conditioning Software Package 2-Place
303411-CU-4-8	**Fear Conditioning Control Unit 4-Place** for control of up to 4 FCS Measuring Units **Requires**: o 303411-SW-4 Fear Conditioning Software Package 4-Place
Control Unit Options	***These options have to be ordered together with the system. Retrofitting of existing systems is not possible***
303411-CU-STIM-NOISE 	**Option Stimulus Noise** An additional noise channel in the control unit allows the application of noise (white noise) as a stimulus signal as an alternative to sine sound in parallel to continuous background noise. The stimulus noise is software-controlled, continuous background noise is manually switched on and off at the control unit front panel. The noise intensity is adjustable independently in both noise channels (identical for all connected boxes, max. 100dB).
303411-CU-SHOCK-1.55	**Option Shock Amplitude 1.55mA** In contrast to the standard shocker (max. amplitude 3.1mA in 0.1mA steps), current

	strengths up to a maximum of 1.55mA are possible with this option. The current strength can here be set in steps of 0.05mA via the software.
303411-CU-TRIGGER	**Option Trigger Package** This option extends the control unit by an additional multi-trigger output, with the help of which information about the status of the experiment can be transferred to an external data acquisition system (e.g. electrophysiology) in real time. A total of 6 signals is available in 6 separate channels: Experiment ON, Experiment OFF, Noise ON, Noise OFF, Shock ON, Shock OFF. As the start and finish of each part appear in different output channels if individual signals were to fail this prevents displacement of the signals and therefore misinterpretation of the information. The signals are always TTL signals 1ms long (other specifications on request). The output socket is customer-specifically configured.
303411-CU-FREQ-FIX	**Option Toggle Switch Sine Frequency** This switch can be used to switch to a present fixed frequency extremely rapidly. Standard frequency: 10 kHz. If a different frequency is required (2...20 kHz ADVANCED SMALL resp. 2…25 kHz ADVANCED LARGE) please mention the frequency in your order.
Software Package	The software package is used to control the experiment in **N** fear conditioning measuring unit and to collect, display and evaluate the measured data. It allows the free definition of stimulus sequences in trace and delay conditioning procedures. Depending on the hardware configuration the following parameters are software-controlled: o Continuous house light (On/Off) o Continuous background noise (On/Off) o Stimulus sound (duration, interval frequency) o Stimulus noise (duration, interval frequency) o Stimulus light (duration) o Shock (duration, amplitude, constant or pulsed). The analysis includes: raw data, activity graph, spacial analysis graph, results tables, event monitor tables. Export files can be generated for import into standard statistical and spreadsheet packages. The software also allows to evaluate Light/Dark experiments.
303411-SW-1	**Fear Conditioning Software Package 1-Place** for WINDOWS **Requires**: o 303411-CU-1 Fear Conditioning Control Unit
303411-SW-2	**Fear Conditioning Software Package 2-Place** for WINDOWS **Requires**: o 303411-CU-2 Fear Conditioning Control Unit
303411-SW-4	**Fear Conditioning Software Package 4-Place** for WINDOWS **Requires**: o 303411-CU-4 Fear Conditioning Control Unit

** It is also possible to order control unit and software package for a larger number of measuring units than number of measuring units actually delivered.*

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.

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