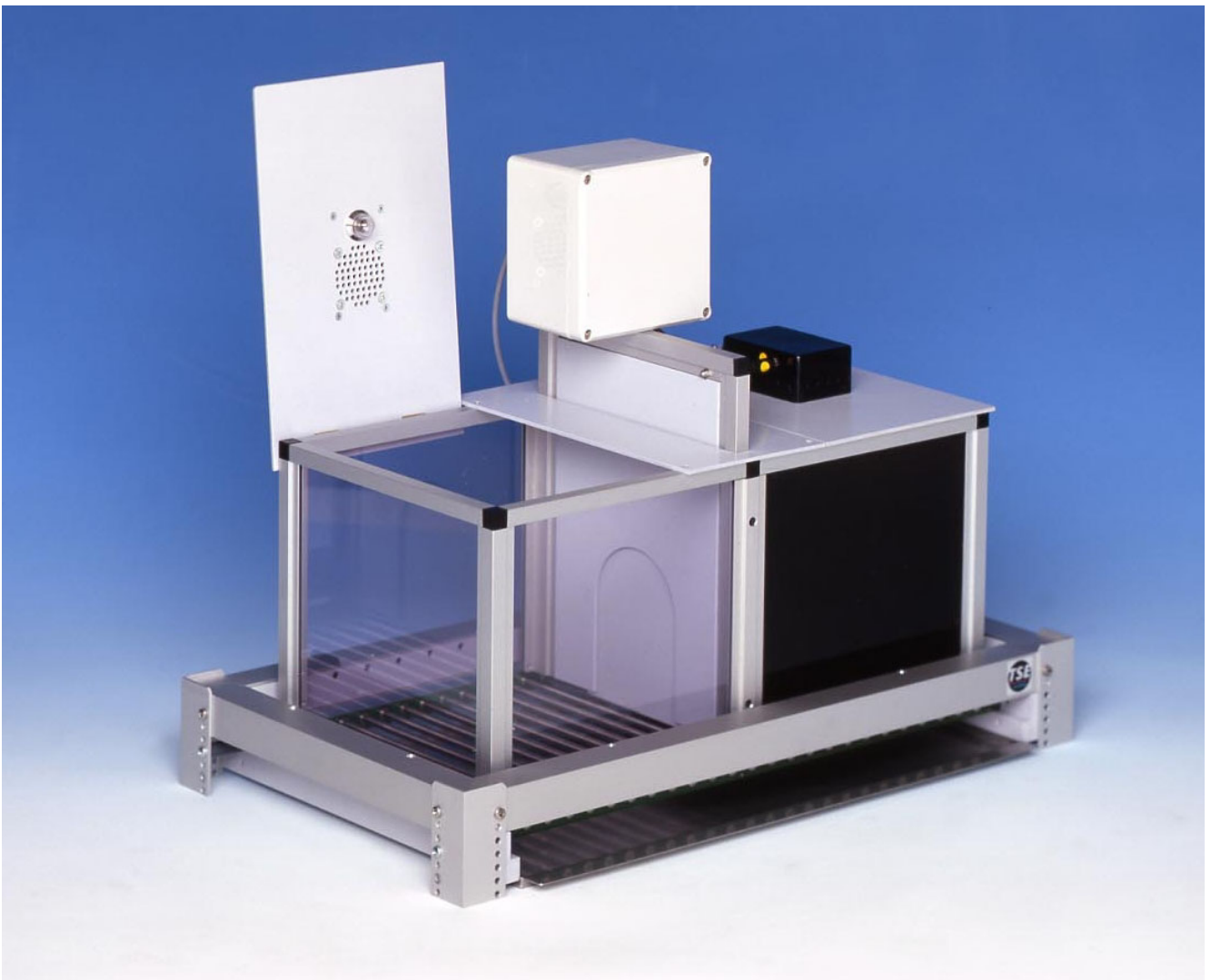# TSE Passive Avoidance System "Basic"

Flexible Test System for Rats and Mice

Passive Avoidance Basic Oct2005.DOC

◆ TSE Passive Avoidance System "Basic"

Overview

Passive Avoidance Box – *Rat* configuration

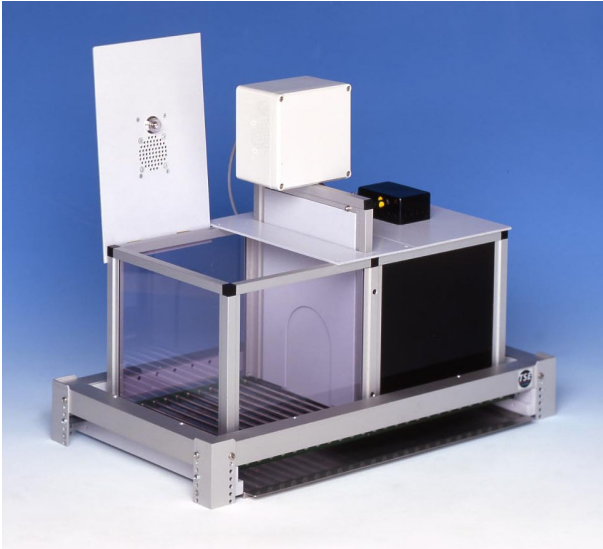The **TSE Passive Avoidance System "Basic"** allows standard passive avoidance experiments ("Step-Through" avoidance) to be carried out with small laboratory animals.

It consists of the following components:

- ◆ up to 4 test boxes *for rats or mice*
- ◆ a control unit
- ◆ special interface to be built into an IBM compatible computer
- ◆ Passive Avoidance software for Windows
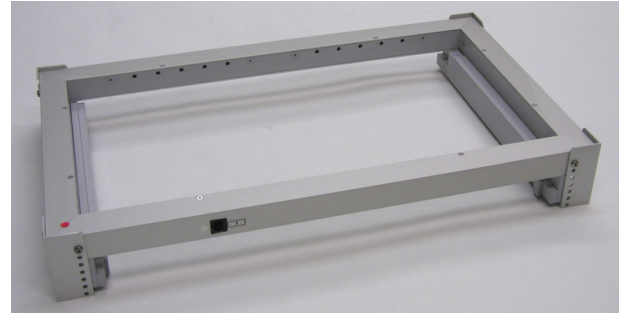- ◆ *optional* housings and other accessories

The test boxes

Passive Avoidance Box – *Rat* configuration

A test box comprises:

1. Base construction - the so-called frame

The size of the frame depends on the animal species. Animal position is monitored with infra-red light barriers scanned with high frequency. The light is invisible to the animal. Each light-barrier consists of one infra-red transmitting and one receiving sensor. These are mounted into transmitter and receiver strips. Movement is recorded in **one** dimension (X-coordinate).

Mouse:	2 x 7 light barriers - 14mm apart
Rat:	2 x 7 light barriers - 28mm apart

2. Removable stainless steel grid
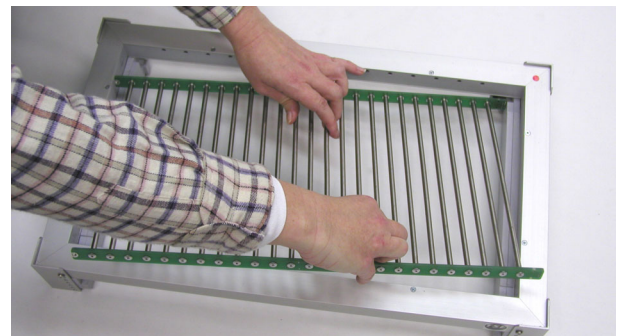
…for delivering a foot shock (US) of defined duration and intensity. Both halves of the grids are electrically independent units - in the standard system shock is available in the dark compartment only. A shocker module for the bright compartment is *optional*.

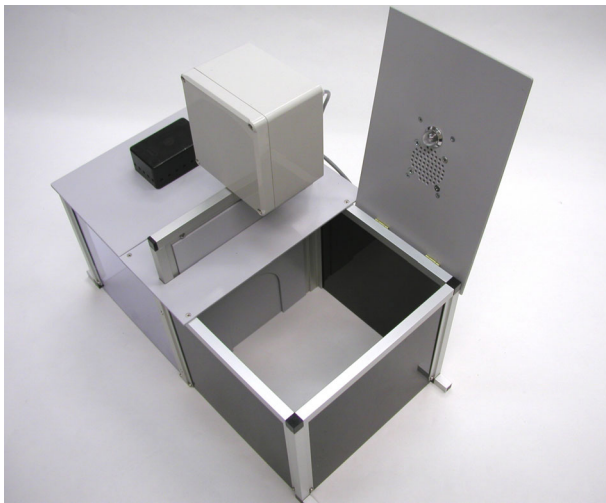|	
Mouse:	Bars with 4mm diameter
	8.9mm distance between rod centers
Rat:	Bars with 6mm diameter
	19.5mm distance between rod centers

A PVC cover plate for the grid located in the bright compartment is included in the scope of supply.

3. Removable feces tray
… to be placed below the sensor frame.

4. Passive Avoidance arena with lid

… to be placed inside the sensor frame. Rat and mouse arenas are available:

Mouse:	280 x 155 x 160mm (LxWxH) compartment width 140mm
Rat:	485 x 230 x 205*mm (LxWxH) compartment width 242mm *270mm optional*

The arena is divided into 2 compartments by an automatic door that can be opened and closed by the software.

|	
Mouse:	37 x 40mm opening (WxH)
Rat:	90 x 115mm opening (WXH)

The arena is equipped with clear walls on one side and with dark walls on the other side. The dark material is permeable to infra-red light.

The animal is placed in the selected compartment from above. Each compartment is equipped with its own lid. The lids feature a software-controlled light (constant illumination). Each lid also contains

a loudspeaker to deliver background noise if desired (1 common sound channel; optional audio/noise generator required).

Other components

The control unit provides the connection between the boxes and the computer. It contains the electronics for the location sensors and for controlling the stimulus components. The standard unit controls up to 4 boxes and features:
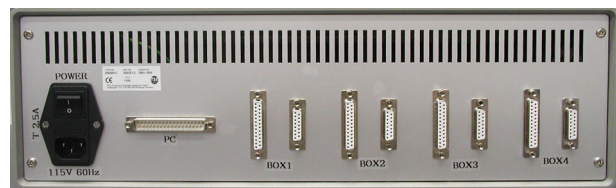
- Light controller for compartment lights; switched ON/OFF via software.
- A shocker/scrambler. One shocker per box is standard to apply a shock to the grid in the dark compartment. The microprocessor controlled current generator with integrated current flow detector ensures current with a high degree of accuracy. Amplitude is software-adjustable in steps of 0.1mA (max. 3.1mA), pulsating or constant.
- An audio/noise generator (optional) for generating continuous background noise with adjustable amplitude, switched on/off by the user before/after the experiment (optional control elements on the control unit front panel).

The control interface (PCI slot) is designed to be built into an IBM-compatible computer. The control unit is connected to this interface.

The Passive Avoidance software controls the whole system and records, analyzes and stores the measuring data.

An IBM compatible computer is required to run the system. Operating system: Windows 98, 2000, XP.

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

Options

◆ In the standard Passive Avoidance configuration shock is given in the dark compartment only. With an additional shocker module a shock can also be given in the bright com-

partment in order to signal the animal to leave the bright compartment after a specific time has elapsed.

◆ The standard shocker allows to adjust the shock amplitude in steps of 0.1mA. If smaller steps are required the shocker is available with a maximum amplitude of 1.5mA – this allows steps of 0.05mA to be applied.

◆ An air puff set can be included in the setup. Air puffs can then be used an an alternative to foot shock.

Box in housing – shown without door

◆ The control unit can be equipped with a TTL output connector for each box. The signal is switched on/off when the experiment is started/stopped. This allows to trigger external hardware in order to achieve common timing (e.g. when the animal´s heart rate is measured with implanted telemetry transmitters).

◆ The box can be operated in a sound attenuating housing, featuring:

- removable front door with integrated "peep hole" viewer,
- house light, switched on and off manually,
- ventilator in the side wall,
- sliding floor plate for easy removal of test box.

◆ More than 4 boxes can be controlled on request.

System extensions

◆ For "Step-Down" Passive Avoidance experiments a special platform is available that is inserted into the bright compartment.
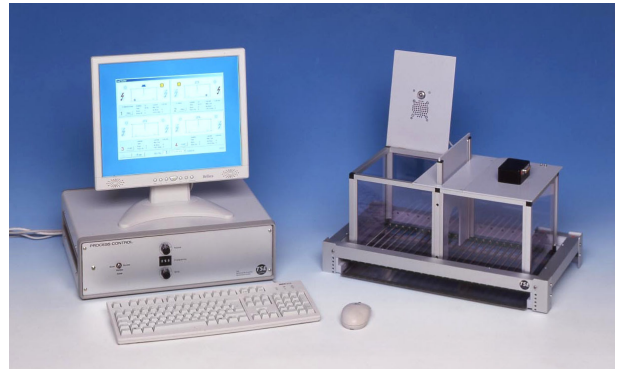
◆ The Passive Avoidance system "Basic" can be easily configured for Active Avoidance experiments, i.e. Shuttle Box experiments, by exchanging the dark walls for clear acrylic walls. 2 shocker modules are required here.

If you are interested in these system extensions please contact us.

TSE Passive Avoidance "Advanced"
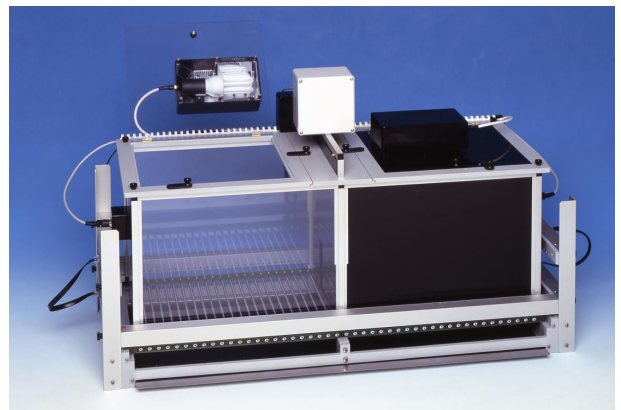
The **TSE Passive Avoidance System** "**Advanced**" is a newly developed test system that has been specifically optimized for Passive Avoidance experiments. It is available as stand-alone rat or mouse system or as a combination system suited for both species.

In contrast to the basic system the new system that features extra-large compartments registers movement in **3** dimensions:

- X- and Y-coordinate for horizontal movement – this allows to generate detailed information about the exploratory behavior of the animal.

- Z coordinate for vertical activity: Rearing (measure of exploration) and Jumping (evaluation of the animal´s shock reaction).

Other features are:

- High-intensity lamp that provides an intensity of up to 1200 Lux in the bright compartment ensuring a high level of aversiveness.

- Specific analysis tables and graphs for detailed analysis of exploration inside the 2 compartments incl. inactivity time, speed, rearing and jumping events.

Please note that this system is available as a 2-place system only and cannot be converted into an Active Avoidance test system.

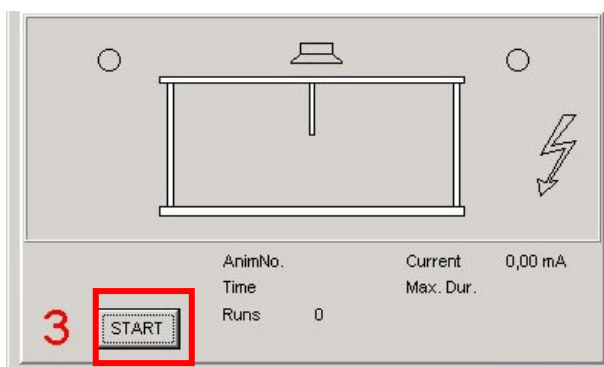***If you are interested in this system please contact us.***

Experimental procedure

Only the Step-Through Passive Avoidance configuration is described in detail here.

A test proceeds according to the following pattern:

◆ test preparation,
◆ test procedure and
◆ subsequent data analysis.
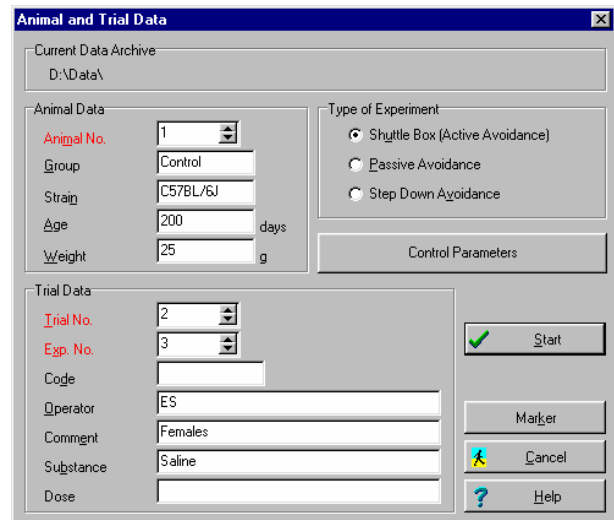
Selecting a box for an experiment

Select the menu item *Experiment* if you want to start the trial in one ore more boxes. A graphical overview appears; this is the so-called "trial monitor".

A box is now selected by clicking on the START button.

The boxes can be independently started at different times from one another. Each box has its own separate run control.

Entering parameters

Before an experiment is started all the parameters which describe the experiment and the animal and which determine the experimental procedure are defined.

An animal is described by providing the following information:

Animal No. / Group / Strain / Age / Weight

An experiment is described by providing the following information:

Trial No. / Exp. No. / Code / Operator / Comment / Substance / Dose

Animal number, *Experiment number* and *Trial number* are obligatory – they can later be used for filtering the stored data files. The input of all other data is optional.

A *Control Parameters* button opens an additional window in which all the information which determines the trial procedure is entered. The contents varies depending on the paradigm selected.

The basic Passive Avoidance task "Step-Through" consists of 2 parts:

1. the **training trial** where the animal is placed into the bright compartment and is given a single foot shock when it enters the dark chamber

2. the **test trial** (memory test) where the latency of the animal to enter the dark chamber where the foot shock (aversive stimulus) was previously delivered is measured. The animal has free access to both compartments. No foot shock is given here.
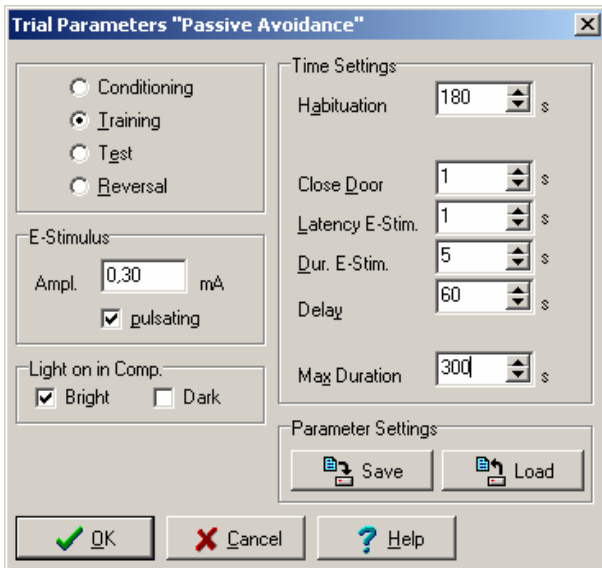
Training

The following time parameters control these experiments. All time entries are made in seconds.

Training only	
Habituation	Exploration time in bright compartment after the experiment has been started. The door is closed during habituation
Close Door	Time from transfer into dark compartment until door is closed. If the animal does not enter the dark side the door will not be closed
Latency E-Stim.	This entry defines the time period between closing the door and the onset of the foot shock (delayed US stimulus)
Dur. E-Stim.	Duration of foot shock. Usually a short shock duration is sufficient
Current Amplitude	Amplitude of foot shock. The amplitude required to induce a clear shock reaction (such as flinching) has to be determined in preliminary tests
Current Type	With the *Pulsating* option the E-stimulus, which is normally applied as a direct current, will be interrupted at regular intervals. On/off periods for the pulsating current are identical.
Delay	Additional time period until the experiment is finished to avoid aversive association with the handling procedure
Max. Duration	If the animal does not change into the dark side the experiment is terminated after this time has elapsed ("Cut-Off Latency")
Light on in Comp. Bright and/or Dark	The light in the selected compartment is switched on when the trial monitor is entered
Test	
Habituation	Exploration time in bright compartment after the experiment has been started. The door is closed

|	during habituation
Max. Duration	After the habituation time has elapsed the door is opened. The trial is ended after this time period has elapsed
Light on in Comp. Bright and/or Dark	The light in the selected compartment is switched on when the trial monitor is entered
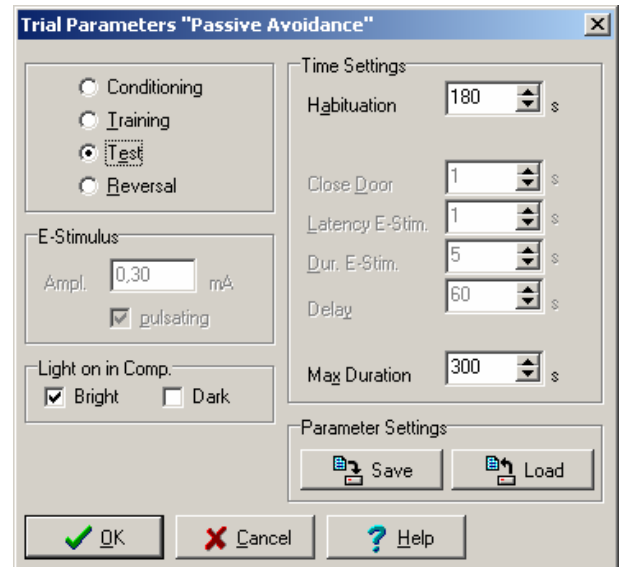
Test

A specific set of control parameters can be stored in setup files for further use.

Further experiments that are available are:

3. The **conditioning trial** corresponds to the conditioning trial of a simple fear conditioning experiment. The animal is placed in the dark compartment and is given a single foot shock. The door is kept close all the time.
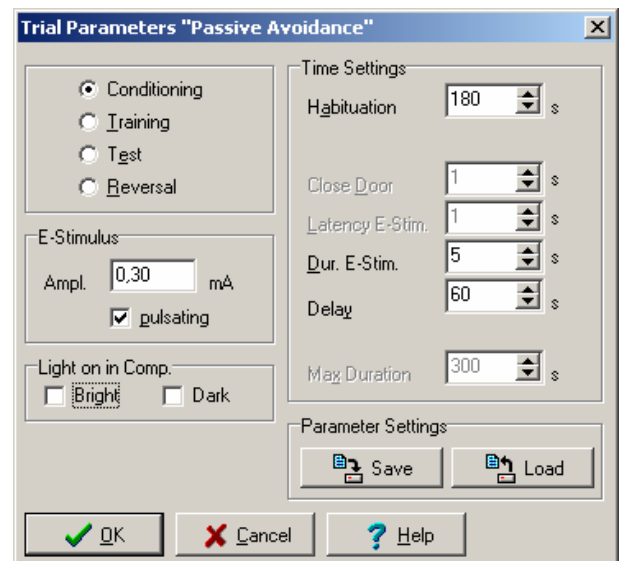

Conditioning
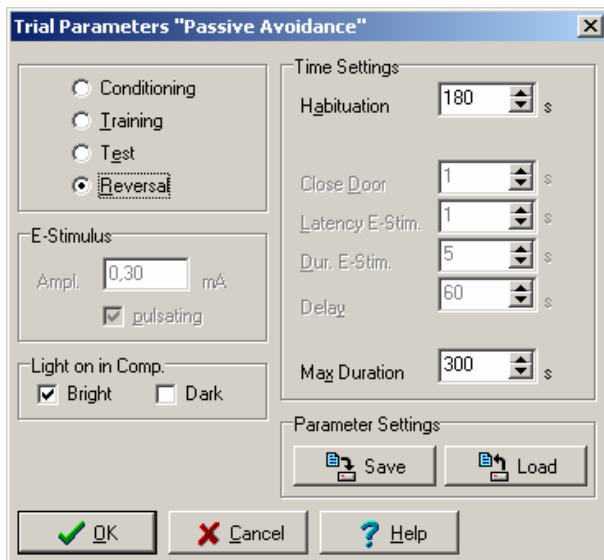
Reversal

4. In a subsequent **reversal** experiment the level of fear conditioning can be evaluated by measuring the latency to leave the dark compartment. No foot shock is given here.

Defining markers

While the experiment is being carried out so-called event markers can be set by the user in order to document particular events. The event markers appear in the analysis tables and export files.
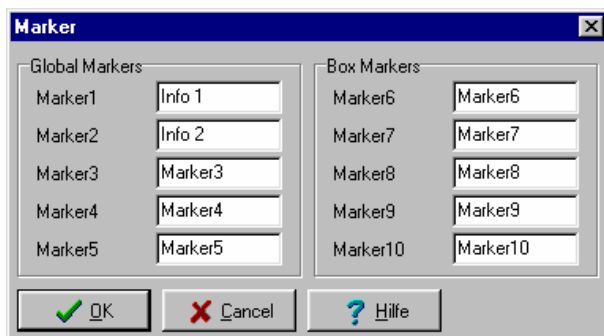

Event markers can be defined *before* the start of the experiment and *while* the experiment is being carried out. 2 categories of markers are available:

− 5 global markers: during the experiment they apply simultaneously to all active boxes.
− 5 box-specific markers characterize events which only affect individual boxes: they are allocated to one specific box only.

Starting the measurement – the trial monitor

When all the parameters have been entered the animal is placed in the box:

− training + test -> in the bright compartment
− reversal + conditioning -> in the dark side
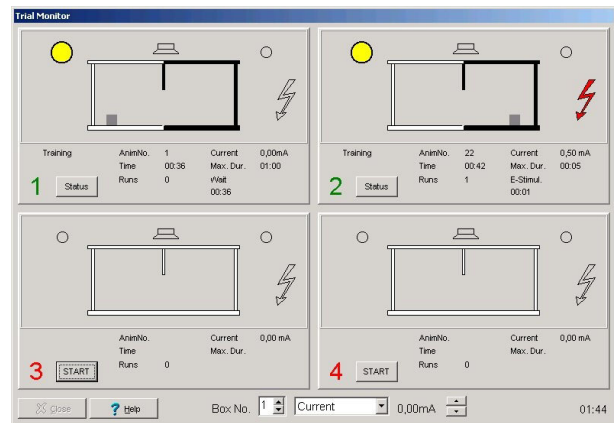
Example for a 2-box-experiment – both boxes are running a training trial

The experiment is started with a mouse-click in a selected box.

The trial monitor shows all relevant information about the actual status of the trial. The location of the animal is represented by a square which alters its position when the animal moves (left, right, center). If loudspeaker, light and E-stimulus functions are activated then the corresponding symbols are highlighted in color.
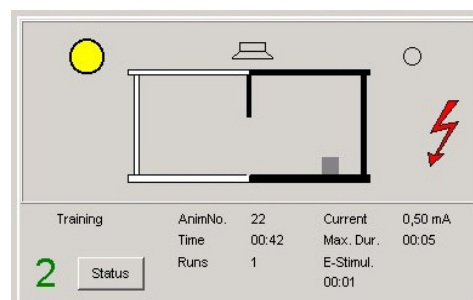
Below the display of the box the following information is output:

− The name of the active trial (e.g. Training)
− As a check the animal number is given
− The amplitude of the current is shown in mA
− The actual trial time is shown in min : s
− Maximum duration of the active phase
− The transfers between compartments (Runs)

– The name of the active phase (e.g. E-stimulus)
– The duration elapsed in the active phase

Example of the experimental sequence in Training and Test trials:

| Training	
	Phase 1 At experiment start the door is closed. The animal is placed in the bright compartment. After the habituation time has elapsed the door is opened. If no habituation time has been defined the experiment is started with the door being opened immediately.
	Phase 2 The animal has entered into the dark side. Since the *Door Closed* parameter has been set to 1 sec the door is closed almost immediately after transfer
	Phase 3 The foot shock with the pre-set amplitude is delivered. After the shock is finished the trial is ended automatically when the defined delay has elapsed
Test	
	Phase 1 At experiment start the door is closed. The animal placed is in the bright compartment. The door opens after the habituation time has elapsed (if defined)
	Phase 2 After the door has been opened transfers are counted. The first entry into the dark compartment is later output in the evaluation table. The trial is finished after the preset duration has elapsed

While the E-stimulus is being applied (only then!) it is possible to manually change the amplitude of the current.
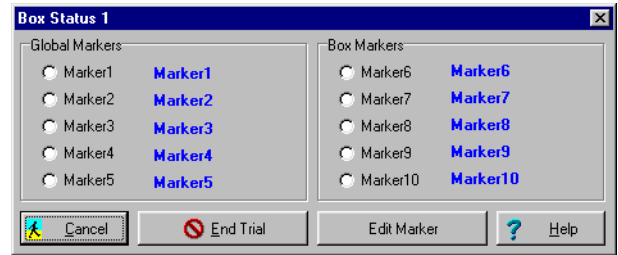
Event markers can be entered in order to record any event that is of importance for the test procedure. They are are added to the data file. Event markers can be defined or changed while the experiment is being carried out.

End of trial

The trial is ended automatically when the termination criterion has been reached. A trial can also always be ended manually by the operator by opening the "Status window" of a specific boy and clicking on the button END TRIAL.
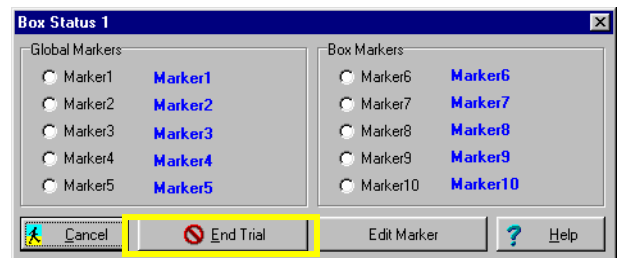
As long as boxes are still active the trial monitor cannot be closed. When the test has been completed in all the boxes the trial monitor can be closed with the CLOSE button which is then active.

Analysis
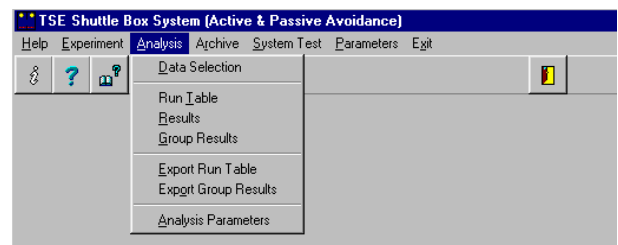
The analysis of stored trial data is carried out in two stages.

The first step is to select the data to be used in the analysis (*Data Selection*).

The data records can be preselected with the aid of a filter in which parameters defined during test preparation are used for filtering criteria; this makes the handling of even large amounts of data easier. Additional analysis parameters allow to adjust the table output to specific requirements of the user.

− Data can be listed as run data (**Run Table**).
− This data is used individually for each experiment to calculate and display different results (**Results Table**).
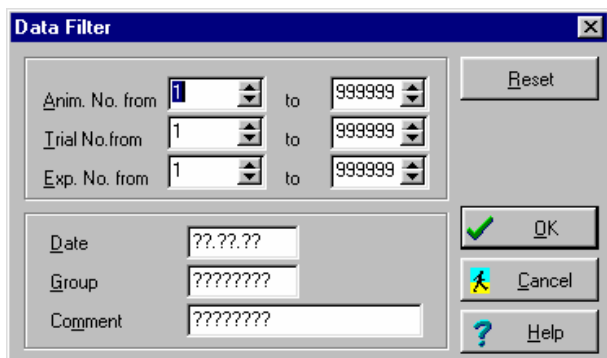− In a group analysis experiments can be compared with each other (**Group Results**).
− Data can be stored in the form of ASCII-compatible files for further processing with statistics programs such as EXCEL.

Run Table

Animal No.	280103
Group	0.2 mA
Strain	C57BL/6J
Age	
Weight	
Trial No.	2
Experiment No.	1
Code	
Operator	
Comment	
Substance	
Dosage	
Start Date	29.01.2003
Start Time	10:13:46
Test	
Habituation	15s
Max. Duration	300s
Light ON in Comp.	Bright

The run table and the results table start with a header which characterizes the data set. The header consists of:

− licensee
− selection of animal and experimental descriptors
− experiment type selected
− control parameters

Run table

The run table provides a chronological list of the events in the box. The table has the following columns:

Time	Time at which event occurred in min:s:1/10s
Event	Description of the event in the box (maximum 2 columns): - Door status at start of experiment - Habituation (*if defined*) + duration - Door status at end of habituation - Position of the animal at end of habituation - Transfer + direction of transfer (into dark or bright compartment) - Door closed - E-Stim. + current type (const. or pulsating) + amplitude in mA - Trial End

00:00:0	Door closed		
00:00:0	Habituation	180s	
03:00:0	Position	Bright	
03:00:0	Door open		
03:21:5	Transfer	Dark	
03:21:8	"Close Door" Position		
03:22:8	Door closed		
03:24:8	E-Stim.	const.	0,20 mA
03:26:8	Delay		
04:26:8	Trial End		

Example: Training

Results table

The results table groups together the run table data in a short form and calculates some result parameters for each data record selected.

No.	Time	Bright	Dark
1	02:06:8	01:51:8	
2	03:55:7		01:48:8
3	04:11:3	00:15:6	
4	05:05:4		00:54:1
5	05:05:5	00:00:1	
6	05:06:0		00:00:4
7	05:15:0	00:08:9	
Total Duration		02:16:5	02:43:4
Total Visits		4	3

Example: Test

The following parameters are listed:

No.	Running number of animal reaction, i.e. transfer
Time	Time at which transfer occurred. The last line corresponds to the time when the trial

	has been finished
Bright	Time that the animal stayed in the bright compartment until transfer occurred
Dark	Time that the animal stayed in the dark compartment until transfer occurred
Total Duration	Total time that the animal spent in the bright/dark compartment
Total Visits	Total number of visits in the bright/dark compartment

Start	Start time
Type	Type of trial (Training, Test, Reversal or Conditioning)
Bright	Number of visits in bright compartment
Dark	Number of visits in dark compartment
TimeB	Total time in bright compartment
TimeD	Total time in dark compartment
Latency	Latency to enter the dark (Training, Test) resp. bright (Reversal) compartment

Group analysis results table

In the group analysis results table the results from several experiments are gathered together in order to make a direct comparison possible.

Group Results

| Anim.	Trial	Exp	Date	Start	Type	Bright	Dark	Time B	Time D	Latency
280103	1	1	28.01.2003	09:06:33	Tr	1	1	00:21:5	01:05:3	00:21:5
280103	2	1	29.01.2003	10:13:46	Te	4	3	02:16:5	02:43:4	01:51:8
280103	3	1	29.01.2003	10:20:02	Re	3	4	00:56:1	04:03:8	01:46:7
280104	1	1	28.01.2003	09:14:02	Tr	1	1	01:06:1	01:05:4	01:06:1
280104	2	1	29.01.2003	10:26:38	Te	1	0	05:00:0	00:00:0	00:00:0
280104	3	1	29.01.2003	10:32:57	Re	0	1	00:00:0	05:00:0	00:00:0
280105	1	1	28.01.2003	09:21:23	Tr	1	1	00:30:9	01:05:9	00:30:9
280105	2	1	29.01.2003	10:39:52	Te	1	0	05:00:0	00:00:0	00:00:0
280105	3	1	29.01.2003	10:46:14	Re	3	3	00:33:8	04:26:1	01:27:5

Each line in the table represents one experiment with one animal. The columns have the following meanings:

Anim.	Animal number
Trial	Trial number
Exp	Experiment number
Date	Start date

Exporting data

In order to use the trial results for further-reaching statistical calculations in statistics packages or spread sheets (e.g. EXCEL) the system offers the possibility of storing run tables and group analysis tables in the form of special export files.

Various settings can be made for the export before carrying out the export itself.
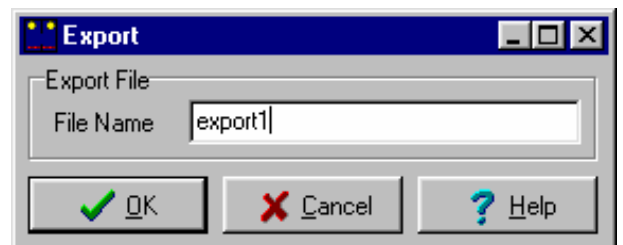
The file name is defined by the user. The file is in the dBase-compatible CSV-format; this is supported by all professional statistics packages.

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