

Startle Response System

Startle reflex & PPI testing system for mice and rats

– Specifications subject to change without notice –

TSE Startle Response System

System description

Startle reflex in small rodents can be easily quantified with our computerized **TSE Startle Response Measuring System**.

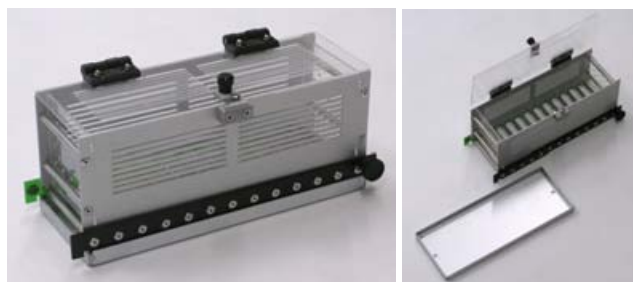
Up to 6 different hardware signals can be controlled independently! Several startle boxes can be operated simultaneously with one computer.



Hardware Components

The system is available for rats and mice and consists of the following components:

- animal restrainers with integrated shockable floor grid that are placed on
- species-specific transducer platforms,
- stimulus generating elements mounted to a “stimulus base unit”,
- sound-attenuated housings,
- a control unit containing the necessary electronics for your choice of stimuli,
- special control interface system for PC or notebook operation and
- the TSE Startle Response software package for Windows.



Rat cage with integrated floor grid & feces tray

The animals are tested restrained in small cages (aluminum, transparent plastics) restricting major movements and exploratory behavior.

Rat and mouse cage models are available. The rat cage can be adjusted to the animal's body size by changing the position of a dividing insert.

Restrainer

Mouse 80 x 40 x 45 mm (LxWxH)

Rat 225 x 80 x 85 mm (LxWxH)

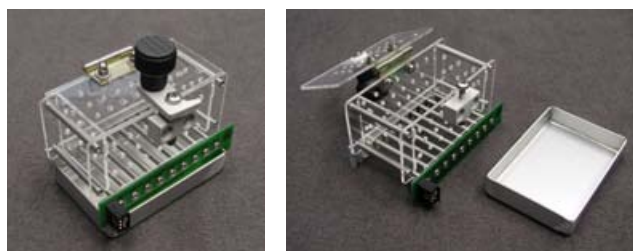
Grid rod floor

Mouse: rod ⌀ 4 mm, distance* 8.9 mm

Rat: rod ⌀ 6 mm, distance* 19.5 mm

* distance rod center to rod center

All cages feature an integrated stainless steel floor grid and a feces tray that can be easily removed for cleaning.



Mouse cage with integrated floor grid & feces tray



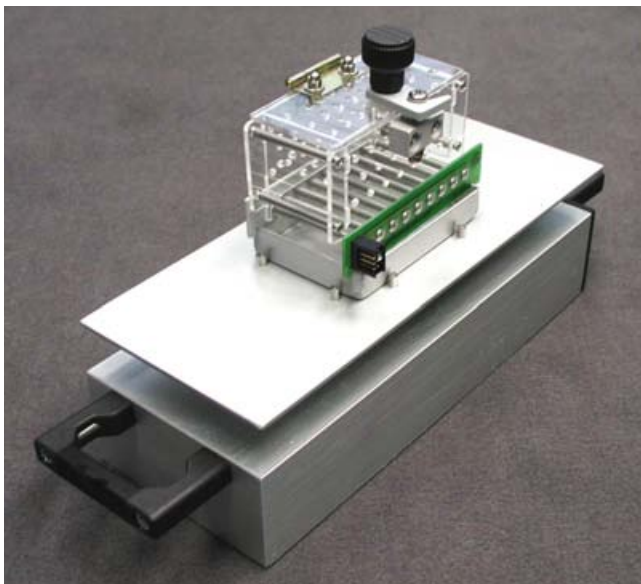
Mouse platform

These cages are placed on species-specific **transducer platforms** mounted on an ultra-stable base construction.

Transducer Platform:

Size 100 x 250 mm
 Maximum Load Mouse 600 g / Rat 3000 g

The highly sensitive transducers allow accurate measurements of the animals' reactions without the need to adjust the setup to the weight of the animal since only dynamic changes are registered!



Mouse cage on mouse platform

Intense short **auditory signals** are generated by means of high-quality high-linearity speakers mounted into the so-called "stimulus base unit".

Both sound with a frequency of up to 25 kHz and white noise can be outputted with user-defined intensity.

Due to the superior performance of the equipment, very rapid rise times – a prerequisite for a stimulus to elicit startle – and a stable amplitude that is independent of the frequency selected are guaranteed!

Technical Data "Auditory Stimuli"

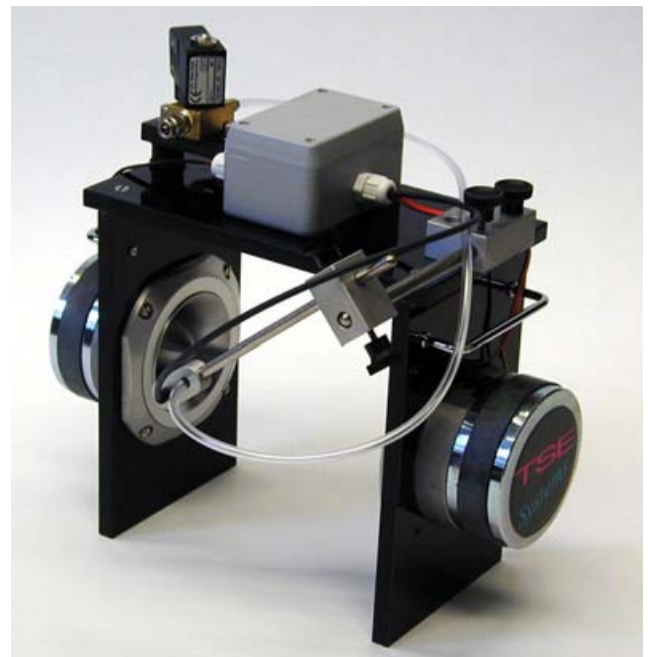
Sine sound 4...25 kHz, max. 130 dB

Noise White noise, max. 130 dB

Both with software-adjustable intensity

In addition to acting as a startling stimulus, continuous white noise can be applied throughout the whole experiment with user-defined intensity. The background noise intensity is set independently from the stimulus noise intensity.

All components for delivery of **air-puffs** (tactile stimuli) via solenoid valves and air delivery tubing is also provided (the user is to provide the air supply and regulator). The air outlet is mounted to a flexible arm that can be brought into any required position.



Stimulus base unit – fully equipped

A **LED** mounted to the same arm allows to apply software-controlled visual stimuli during the trial.



Light & Air Puff Unit

An **electrical stimulus** can be applied through the floor grid in order to evaluate fear-potentiated startle. Usually the foot shock is paired with the signal light.

The control unit is then equipped with a microprocessor-controlled shocker module to produce these foot shocks. The module ensures a constant current with a high degree of accuracy. If the experimenter requires, a pulsating stimulus current can also be applied.

Programmable Shock Generator

Current type constant or pulsating current
 Current strength 0.1...3.1mA (in steps of 0.1mA)

This module always carries out a so-called current flow check, i.e. a check is made if current is really flowing.

Housing Dimensions

Inside 410 x 410 x 410 mm
 Outside 490 x 490 x 490 mm

The housing features a one-way observation window in the front door to provide for monitoring and VCR recording during the experiment. It can be closed if desired. A manually operated house-light is also included in the housing construction.



Process control unit

The control unit provides the connection between the boxes and the computer. It contains all the electronics for controlling the stimulus components and transfers the measuring data to the control interface. The control interface is available for PC or for notebook operation. An IBM-compatible computer (Pentium) is required.



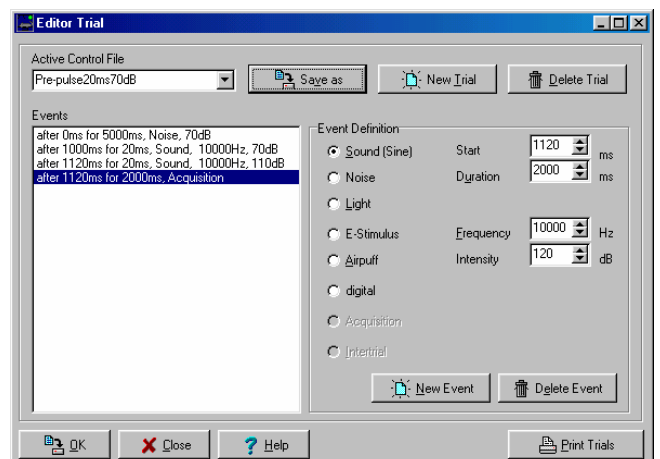
Housing

The whole setup is operated in sound-attenuating isolation chambers (**housing**) equipped with a ventilation fan (can be switched off if necessary) that also provide odor isolation.

Software Control

The comfortable "Startle Response" Windows software that controls the experimental procedure and collects the measuring data is very easy to learn and use. On-line help is provided describing all program functions in detail. The software languages currently available are English and German.

Integrated Editor



Trial Editor

The integrated flexible **editor** allows the quick creation of individual test paradigms.

Trials can be created using the **events** provided. A trial can consist of one event only. Alternatively several events can be combined to user-defined event sequences. The hardware signals currently available are:

<u>Event Type</u>	<u>Adjustable Parameters</u>
Sine Sound	Start, Duration, Intensity (dB), Frequency (Hz)
Noise	Start, Duration, Intensity (dB)
Light	Start, Duration
E-Stimulus	Start, Duration, Intensity (mA), pulsed or constant
Air Puff	Start, Duration
Digital Signal	Start, Duration

Deliver strong or weak stimuli, change their duration and define presentation start time as required. Beginning and end of each event is defined in milliseconds from the start of the trial.

Example of typical settings:

Pre-pulse	10 kHz sound, 20ms, 70dB 100ms before onset of startle stimulus
Stimulus	10 kHz sound, 20ms, 120dB

Background noise can be presented in order to provide a defined acoustic background during the experiment (typical intensity 70 dB).

For assessment of fear-potentiated startle (i.e. the excitatory effect on the startle response) the amplitude and duration of the electrical stimulus is defined here.

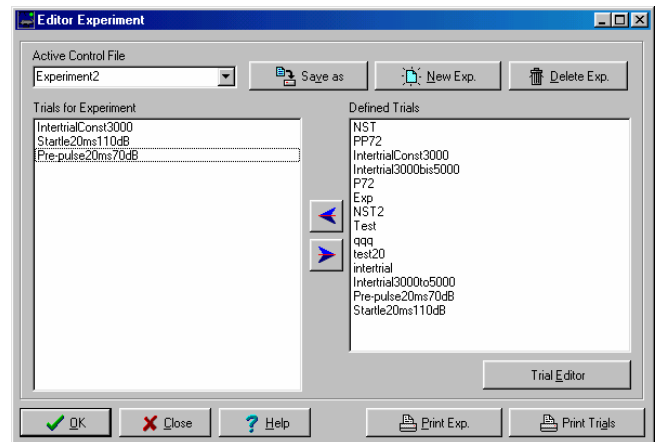
The foot shock may also be paired with a light stimulus of user-defined duration.

The system also provides a TTL signal (digital signal) that can be used to switch on a camera or a video tape recorder or to control additional external devices such as user-specific signal generators.

<u>Event Type</u>	<u>Adjustable Parameters</u>
Acquisition	Start, Duration
Intertrial Interval	Duration (constant or variable)

Data are stored in sampling windows (“Acquisition”) with user-defined onset and length. Usually data storage is started simultaneously with the startling stimulus in order to directly obtain the latency of the

startle reflex in the analysis. Data may also be collected for longer periods depending on the information that is required.



Experiment Editor

Constant or variable inter-trial intervals (ITI) can be defined that are later used to automatically separate trials from each other.

These pre-defined trials are stored in files for future use.

The next step is to combine trials to **experiments**. The same trial can be run repeatedly with constant or variable inter-trial intervals. Different trials can be combined in order to create complex designs.

Whether you want to apply

- a startling stimulus only to measure habituation, i.e. the reduction of the SR magnitude,
- pre-pulses only,
- pre-pulse + startle stimulus to measure inhibitory effects on the startle response (pre-pulse inhibition (PPI)) or
- output no stimulus at all to determine baseline activity,

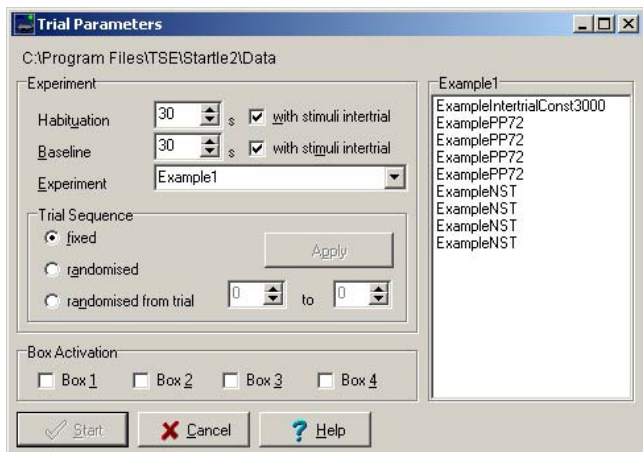
- create simple experiments or complex paradigms to meet your individual requirements.

All experimental designs are easily stored in control files for future use.

Preparing for an Experiment

Trial preparation includes definition of a **habituation time**, if required. A **baseline determination** phase allows to collect baseline activity of the animal before the experiment is started - this baseline activity can

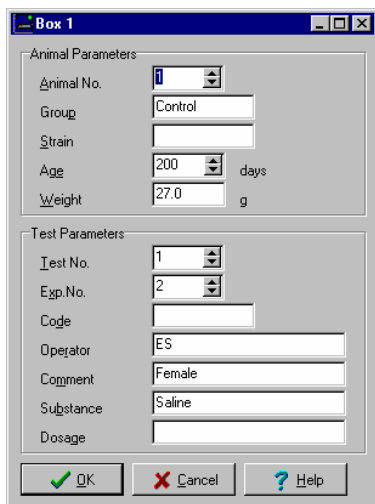
later be used to automatically set the trigger threshold for analysis.



Trial Preparation

The **experimental file** to be run is selected now. The sequence of trial presentation can be fixed or randomised. It is also possible to select a group of trials for randomisation.

In order to characterize the experiment various entry fields are available that are filled in before the experiment is started. These identifiers later allow easy searching through the data base and are also outputted in the protocol.



Descriptive Entries

After all data have been entered, the animals are placed into the boxes and the boxes closed. The experiment is started simultaneously in all units that are connected up by pressing a single key.

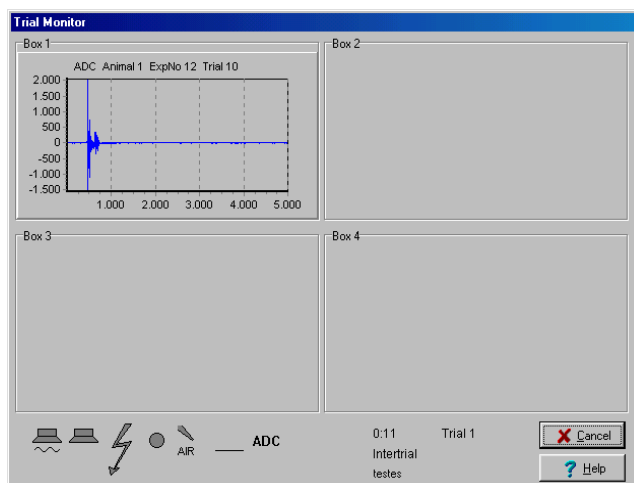
The Running Experiment



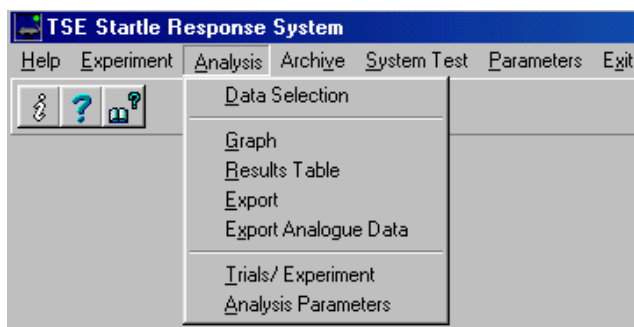
Now exposure to stimuli is initiated according to the control file loaded. The so-called "trial monitor" allows a rapid overview throughout the course of the experiment by displaying active stimuli with colored symbols, thus allowing the status of all connected boxes to be seen at a glance.

Startle movements by the animal are picked up by the transducer, amplified and converted into a digital signal with 12-bit resolution. The full transducer range is available for response detection!

Immediately after each trial the response waveforms are outputted in a coordinate system with the time in ms as the X-axis and the measured force in ADC units as the Y-axis.



Analysis of Measured Data



Search functions allow the easy selection of data records to be analyzed from the data base. A filter function is provided to facilitate data management.

Data Selection						
Available Data Records C:\Programme\TSE\Startle2.01\Data						
Animal	No.	Group	Trial	Exp.	Date	Comment
1	45		3	5	23.05.01	
1	46		3	5	23.05.01	
3	47		3	5	23.05.01	
4	48		3	5	23.05.01	
5	49		3	5	23.05.01	
5	50		3	5	23.05.01	
7	51		3	5	23.05.01	
7	52		3	5	23.05.01	
2	37		4	3	05.06.01	nach 1 Woche
9	54		3	5	23.05.01	

Selected Data Records (3)						
Animal	No.	Group	Trial	Exp.	Date	Comment
3	47		3	5	23.05.01	
4	48		3	5	23.05.01	
5	49		3	5	23.05.01	

Data Selection

A protocol is available giving details on the course of each experiment.

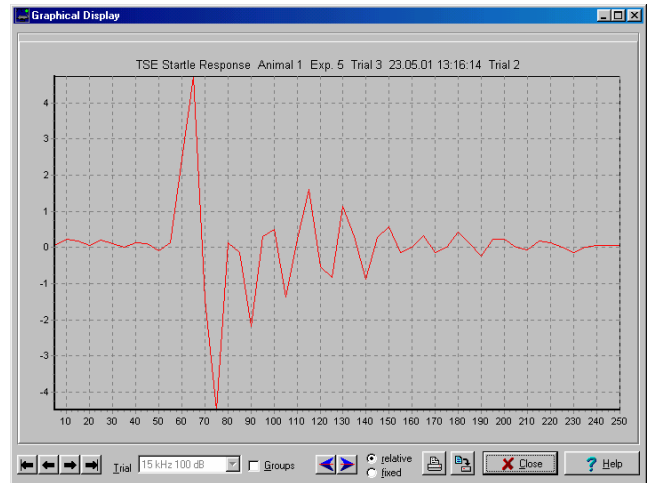
Trials/Experiment	
25.09.2001	
Animal No.	6
Trial No.	4
Exp. No.	3
Start Time	05.06.01 10:47:12
Group/Strain Code	
Operator	
Comment	nach 1 Woche
Substance	
IntertrialConst	8000
from 8000ms	to 8000ms
Intertrial	
habi 1	
after 0ms	for 300000ms
after 0ms	for 300000ms
Noise	60dB
Acquisition	
Rauschen 120 dB	
after 0ms	for 250ms
after 50ms	for 50ms
Acquisition	
Noise	10dB
habi 1	
Rauschen 120 dB	
Rauschen 120 dB	
Rauschen 120 dB	
Rauschen 120 dB	

A **graph** can be called up displaying the storage events that have been defined in the experimental paradigm.

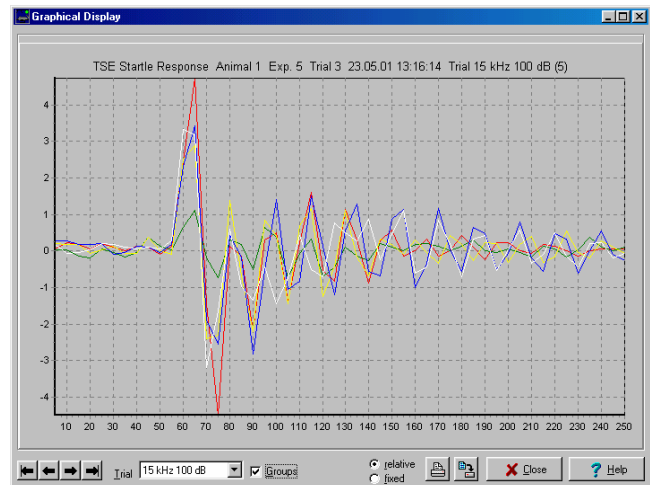
The animal's reaction is displayed in a coordinate system with the time in ms as the X-axis and the change in force picked up by the sensor in grams as the Y-axis.

One graph corresponds to one acquisition event, i.e. one complete trial or one section out of a trial. If more than 1 storage event has been defined in the experiment than these acquisition phases can be called up one after another with the arrow keys provided.

In order to facilitate comparison of data records the graph can be switched to the absolute representation where the Y-axis is displayed with a user-defined end point.



1-trial-display



Trial Overlay (Response to Startle Stimulus)

If several identical trials were run in the experiment then these trials can be displayed in the same coordinate system for direct visual comparison (overlay display).

Whether the response magnitude is increased (shock sensitization) or decreased (e.g. due to habituation or pre-pulse inhibition) or whether the latency changed over the course of the experiment can then be seen at a glance.

The **results table** lists for each storage period:

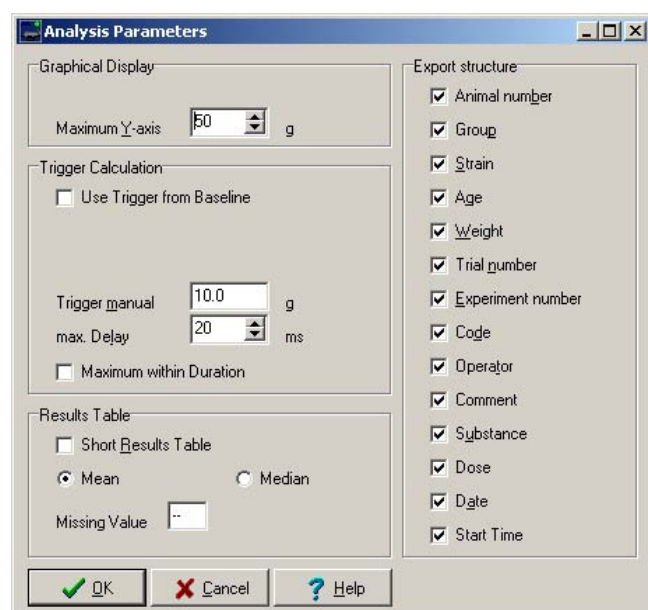
- the startle latency in milliseconds (**Reaction ms**), i.e. the time after the start of the storage interval when the startle reaction occurred,
- the **duration** of the startle reaction (in ms),
- the maximum amplitude recorded in the acquisition window in grams (**Maximum g**) and
- the latency of this maximum amplitude (**Maximum ms**)

Animal No. 1
 Trial No. 3
 Exp. No. 5
 Start Time 23.05.01 13:16:14
 Trigger 3,0 g
 max. Delay 20 ms

Trial	Reaction ms	Duration ms	Maximum g	Maximum ms
dummyton				
1	5	5	52,3	5
Mean	5,0	5,0	52,3	5,0
15 kHz 100 dB				
2	65	15	4,7	65
4	0	0	1,1	65
6	0	0	2,9	65
8	65	5	3,4	65
10	60	15	3,3	60
Mean	63,3	11,7	3,1	64,0
schock 0,6 mA				
3	65	145	11,2	165
5	65	140	17,9	95
7	65	145	11,8	95
9	0	0	1,9	215
11	0	0	0,7	55
Mean	65,0	143,3	8,7	125,0

Identical trials are grouped together for mean value calculation.

Calculation of the startle reaction is influenced by a **trigger** threshold that can be set by the operator: only reactions above this threshold are taken into account.



Alternatively the trigger threshold that has been calculated automatically by the system during the calibration phase can be used for analysis.

Time calculations in this table always refer to the beginning of the storage window. Therefore if the storage window has been chosen to start with the onset of the acoustic startle stimulus, the values outputted in the table represent the absolute distance between stimulus start and startle reaction.

The reaction duration depends on the parameter "max. Delay" that is also defined by the operator. After falling below the trigger threshold a reaction is only then interpreted as being finished if, during a following time window with the length "max. Delay", this threshold is not exceeded again. This calculation allows the summarization of a sinusoidal waveform into one reaction event.

The results table can also be outputted in a shortened form that lists the mean values for each trial. Since mean value calculation takes only those trials into account where the reaction has exceeded the trigger threshold the percentage of these trials as compared to the total trial number is also given.

Trial	Reaction ms	Duration ms	Maximum g	Maximum ms
habi 1	5,0	5,0	58,6	5,0
	100,0%	100,0%		
Rauschen 120 dB	76,8	26,8	4,5	93,8
	68,0%	68,0%		

Data Export

All measuring data can also be converted into ASCII files for further-reaching complex statistics with statistical, database or spreadsheet programs.

Anim	Trial	Exp	Date	Start	TrigG	PauseMS	No	Trial	RTMS	DurMS	MaxG	MaxMS
2	4	3	05.06.01	10.04.24	1	20	1	habi 1	5	5	51,7	5
2	4	3	05.06.01	10.04.24	1	20	2	Rauschen 120 dB	70	105	3,1	165
2	4	3	05.06.01	10.04.24	1	20	3	Rauschen 120 dB	75	5	2,3	110
2	4	3	05.06.01	10.04.24	1	20	4	Rauschen 120 dB	65	155	5,6	80
2	4	3	05.06.01	10.04.24	1	20	5	Rauschen 120 dB	60	90	4	75
2	4	3	05.06.01	10.04.24	1	20	6	Rauschen 120 dB	60	115	2,5	110
2	4	3	05.06.01	10.04.24	1	20	7	Rauschen 120 dB	60	160	7,9	75
2	4	3	05.06.01	10.04.24	1	20	8	Rauschen 120 dB	75	125	3,3	90
2	4	3	05.06.01	10.04.24	1	20	9	Rauschen 120 dB	60	125	3,4	75
2	4	3	05.06.01	10.04.24	1	20	10	Rauschen 120 dB	150	15	1,1	160

If, for example, pre-pulse inhibition is to be analyzed the degree of response inhibition can be easily calculated from these files using the initial startle as a reference.

Adjustable export parameters allow the adaptation of the file structure to the individual requirements of the user.

All graphs can be stored as bitmap-files for integration into word processing or graphics programs. The graph can also be exported as an ASCII file listing the force values for each individual sampling point for further graphical and statistical evaluation in external program packages.

Anim	Trial	Exp	Date	Start	No	Trial	MS	ValueG
2	4	3	05.06.01	10:04:24	1	habi 1	0	51,7
2	4	3	05.06.01	10:04:24	2	habi 1	5	-0,1
2	4	3	05.06.01	10:04:24	3	habi 1	10	-0,1
2	4	3	05.06.01	10:04:24	4	habi 1	15	-0,1
2	4	3	05.06.01	10:04:24	5	habi 1	20	0
2	4	3	05.06.01	10:04:24	6	habi 1	25	0
2	4	3	05.06.01	10:04:24	7	habi 1	30	0,1
2	4	3	05.06.01	10:04:24	8	habi 1	35	0,1
2	4	3	05.06.01	10:04:24	9	habi 1	40	0,3

An integrated **function test** allows all components to be tested by the user in order to check whether the system is functioning properly.

A variety of **program parameters** allow the adjustment of the system to the specific hardware requirements.

In order to compensate any minor variations in loudspeaker frequency response a **correction function** can be activated that will be effective during the experiment.

For very small reactions the **signal amplification** can be increased in order to achieve higher amplitude resolution.

Other Functions

System Test

Stimuli

Air Light E-Stim Current: 0.2 mA

Sound Intensity: 100 dB Frequency: 10000 Hz

Noise Intensity: 100 dB

Loudspeaker calibration

Max. Intensity Sound 10kHz: 110.0 dB Calibration sound 10kHz

Max. Intensity Noise: 110.0 dB Calibration noise

Attenuation for calibration:

20 dB 30 dB 40 dB 50 dB

ADC

	ADC	ADC min	ADC max	Value	Time ADC=
Box 1	297	-597	1000	10.0	0.0
Box 2	298	-598	1000	10.0	
Box 3	299	-599	1000	10.0	
Box 4	300	-596	1000	10.1	

Ampl.=1 Reset Graph

Interface Version

Version DLL ME300 00000000 Version Kithara 00006050
Version modul ME300 00000000 PC-Timer Frequency 1211.931 MHz

Time Control

Sampling Interval SET = 2ms ACT = **1.99970ms**

Close Help 1:20

Partial list of users

- Abbott GmbH & Co. KG, Ludwigshafen, Germany
- Altana Pharma AG, Barsbüttel, Germany
- Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany
- EGIS Pharmaceuticals Ltd., Budapest, Hungary
- Fraunhofer Institut für Toxikologie & Exp. Medizin, Hannover, Germany
- Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
- Leibniz-Institut für Neurobiologie, Magdeburg, Germany
- Max-Planck-Institut für Experimentelle Medizin, Göttingen, Germany
- Max-Planck-Institut für Hirnforschung, Frankfurt, Germany
- Medizinische Hochschule Hannover – MHH, Hannover, Germany
- Merck KGaA, Darmstadt, Germany
- NeuroSearch A/S, Ballerup, Denmark
- NISAD Neurosc.Inst.Schizophrenia & Allied Disord., Darlinghurst Sydney, NSW, Australia
- Nofer Institute of Occupational Medicine, Lodz, Poland
- Organon Laboratories Ltd., Motherwell, Lanarkshire , Great Britain
- Orion Cooperation - Orion Pharma, Turku, Finland
- Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany
- Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany
- Rijksuniversiteit Groningen, Groningen, The Netherlands
- Ruhr-Universität Bochum, Bochum, Germany
- Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany
- Suven Life Sciences Limited, Hyderabad, India
- Universität Bremen, Bremen, Germany
- University of Kuopio, Kuopio, Finland
- U.S. Environmental Protection Agency – EPA, Research Triangle Park, NC, USA

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- **Schneider M, Drews E, Koch M.** Behavioral effects in adult rats of chronic prepubertal treatment with the cannabinoid receptor agonist WIN 55,212-2. *Behavioural Pharmacology* 2005; 16: 447-53.
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- **Schneider M, Koch M.** Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. *Neuropsychopharmacology* 2003; 28: 1760-9.
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Ordering Information

252000-BOX	<p>Startle-Response-Housing For 1 animal for connecting of 1 cage (rat or mouse)</p> <p>Consisting of sound attenuating housing with built in ventilator, house light, window, stimulus base unit (for connecting loudspeaker (audio), air puff, light, etc.), and all connectors.</p>
252000-C/01	<p>Startle Control-Unit 1-Place For stimulus and data acquisition</p> <p>For connecting of 1 startle-response cage 252000-CAG-M or 252000-CAG-R, extendable. Complete and comprising (for connecting to PC): control-unit for stimulus and data acquisition for connecting audio generator and shocker for electrical stimulus, including special interface.</p>
252000-C/02	<p>Startle Control-Unit 2-Place For stimulus and data acquisition</p> <p>For connecting of up to 2 startle-response cages 252000-CAG-M or 252000-CAG-R, extendable. Complete and comprising (for connecting to PC): control-unit for stimulus and data acquisition for connecting audio generator and shocker for electrical stimulus, including special interface.</p>
252000-C/03	<p>Startle Control-Unit 3-Place For stimulus and data acquisition</p> <p>For connecting of up to 3 startle-response cages 252000-CAG-M or 252000-CAG-R, extendable. Complete and comprising (for connecting to PC): control-unit for stimulus and data acquisition for connecting audio generator and shocker for electrical stimulus, including special interface.</p>
252000-C/04	<p>Startle Control-Unit 4-Place For stimulus and data acquisition</p> <p>For connecting of up to 4 startle-response cages 252000-CAG-M or 252000-CAG-R, extendable. Complete and comprising (for connecting to PC): control-unit for stimulus and data acquisition for connecting audio generator and shocker for electrical stimulus, including special interface.</p>
252000-C/05	<p>Startle Control-Unit 5-Place For stimulus and data acquisition</p> <p>For connecting of up to 5 startle-response cages 252000-CAG-M or 252000-CAG-R, extendable. Complete and comprising (for connecting to PC): control-unit for stimulus and data acquisition for connecting audio generator and shocker for electrical stimulus, including special interface.</p>
252000-C/06	<p>Startle Control-Unit 6-Place For stimulus and data acquisition</p> <p>For connecting of up to 6 startle-response cages 252000-CAG-M or 252000-CAG-R, extendable. Complete and comprising (for connecting to PC): control-unit for stimulus and data acquisition for connecting audio generator and shocker for electrical stimulus, including special interface.</p>
252000-C/08	<p>Startle Control-Unit 8-Place For stimulus and data acquisition</p> <p>For connecting of up to 8 startle-response cages 252000-CAG-M or 252000-CAG-R, extendable. Complete and comprising (for connecting to PC): control-unit for stimulus and data acquisition for connecting audio generator and shocker for electrical stimulus, including special interface.</p>

252000-C/10	<p>Startle Control-Unit 10-Place For stimulus and data acquisition</p> <p>For connecting of up to 10 startle-response cages 252000-CAG-M or 252000-CAG-R, extendable. Complete and comprising (for connecting to PC): control-unit for stimulus and data acquisition for connecting audio generator and shocker for electrical stimulus, including special interface.</p>
252000-C/12	<p>Startle Control-Unit 12-Place For stimulus and data acquisition</p> <p>For connecting of up to 12 startle-response cages 252000-CAG-M or 252000-CAG-R, extendable. Complete and comprising (for connecting to PC): control-unit for stimulus and data acquisition for connecting audio generator and shocker for electrical stimulus, including special interface.</p>
252000-C/16	<p>Startle Control-Unit 16-Place For stimulus and data acquisition</p> <p>For connecting of up to 16 startle-response cages 252000-CAG-M or 252000-CAG-R, extendable. Complete and comprising (for connecting to PC): control-unit for stimulus and data acquisition for connecting audio generator and shocker for electrical stimulus, including special interface.</p>
252000-CAG-M	<p>Startle Reflex Cage Mouse, shockable For adaption to platform</p> <p>Made from acrylic, stainless steel and aluminium.</p>
252000-CAG-R	<p>Startle Reflex Cage Rat, shockable For adaption to platform</p> <p>Made from acrylic, stainless steel and aluminium.</p>
252000-PF-M	<p>Startle Reflex Platform for Mouse Complete with highly sensitive transducer</p> <p>For registration of the response.</p>
252000-PF-R	<p>Startle Reflex Platform for Rat Complete with highly sensitive transducer</p> <p>For registration of the response.</p>
252000-S	<p>Software-Package Startle-Response for WINDOWS For up to 4 Startle-Response Boxes, extendable.</p>
252000-S/E-04>08	<p>Software-Package Startle-Response for WINDOWS Extension 4 to 8 Startle-Response Boxes, extendable.</p>
252000-S/E-08>12	<p>Software-Package Startle-Response for WINDOWS Extension 8 to 12 Startle-Response Boxes, extendable.</p>
252000-ST/ANG	<p>Audio / Noise Generator Required: 1 pc. per system.</p>
252000-ST/AP	<p>Air Puff (set). Required: 1 set for each box.</p>
252000-ST/LI	<p>Stimulus-Lamp. Required: 1 lamp for each box.</p>
252000-ST/LS	<p>High-frequency Loudspeaker (pair) For audio stimulus Required: 1 pair for each box.</p>
252000-ST/SHOCK	<p>Shocker for electrical stimulus To be built into the control unit 252000-C. Standard output 0.1 ... 3.1 mA, constant or pulsating, up to 4.5 mA on request. Required: 1 pc. for each box.</p>

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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