Panlab
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USER MANUAL

PACKWIN 2.0.09

TABLE OF CONTENTS

1 INTRODUCTION 1

1.1 What's New in PACKWIN 2.0? 1
1.2 A new modular structure 2
1.3 System Packaging 4

1.3.1 Experimental Boxes and Accessories 5
1.3.2 Interface Units 9
1.3.3 Computer 10
1.3.4 The PACKWIN Software & USB Key 11

2 SYSTEM INSTALLATION 12

2.1 Computer Requirements 12
2.2 Box Assembly & Connection to the Interfaces 13

2.2.1 PACKWIN Operant Modules (CS, HO and VT) 13
2.2.2 PACKWIN Freezing and Startle Module (CSFR and CSST) 14

2.3 Set the Link Box Identification Number (Only for Operant Modules) 14

2.3.1 PACKWIN Operant Modules (CS, HO and VT) 14
2.3.2 PACKWIN Freezing & Startle Module (CSFR and CSST) 15

2.4 Install USB Connection Drivers 15

2.4.1 PACKWIN Operant Modules (CS, HO and VT) 15
2.4.2 PACKWIN Freezing & Startle Module (CSFR and CSST) 20

2.5 Install the PACKWIN Software 23

2.5.1 The USB protection key management 24
2.5.2 Evaluating PACKWIN: the trial period 25

2.6 Install the Configuration File 27

2.6.1 PACKWIN Operant Modules (CS, HO and VT) 27
2.6.2 PACKWIN Freezing and Startle Modules (CSFR and CSST) 27

2.7 Experimental File Example 28

2.7.1 PACKWIN Operant Modules (CS, HO and VT) 28
2.7.2 PACKWIN Freezing Module (CSFR) 28
2.7.3 PACKWIN Startle Module (CSST) 28

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2.8 INSTALL THE REMOTE CONTROL 29
2.8.1 CONNECTING THE TELESWITCH 29
2.8.2 STARTING AND STOPPING TRIALS WITH THE TELESWITCH 30
2.9 ADDITIONAL COMPUTER CONFIGURATIONS 30
2.9.1 AUTOMATIC SETTING TO MAXIMUM VOLUME 32
2.9.2 SOUNDS SCHEME DEACTIVATION 33
2.9.3 LOAD CELL CALIBRATION & OPTIMIZATION 33
3 STARTING WITH PACKWIN 42
3.1 STARTING ASSISTANT PANEL 42
3.1.1 CREATING A NEW EXPERIMENTAL FILE 43
3.1.2 CONTINUING AN EXPERIMENT 44
3.1.3 ANALYZING AN EXPERIMENT 44
3.2 PACKWIN MAIN WINDOW 46
3.2.1 TITLE BAR 46
3.2.2 MAIN MENU BAR 46
3.2.3 EXPERIMENTATION ASSISTANT BAR 52
3.2.4 EXPERIMENT IDENTIFICATION BAR 53
4 BOXES DEFINITION 53
4.1 REAL BOXES DEFINITION 54
4.1.1 PACKWIN OPERANT MODULE (CS) 54
4.1.2 PACKWIN OPERANT NINE HOLE MODULE (HO) 59
4.1.3 PACKWIN OPERANT VOGEL TEST MODULE (VT) 61
4.1.4 PACKWIN FREEZING AND STARTLE MODULE (CSFR AND CSST) 63
4.2 VIRTUAL BOXES DEFINITION 69
4.2.1 PACKWIN OPERANT MODULES (CS) 69
4.2.2 PACKWIN OPERANT NINE HOLE MODULE (HO) 70
4.2.3 PACKWIN OPERANT VOGEL TEST MODULE (VT) 70
4.2.4 PACKWIN FREEZING MODULE (CSFR) 71
4.2.5 PACKWIN STARTLE MODULE (CSST) 71
5 BOXES TEST 72
5.1 PACKWIN OPERANT MODULE (CS) 73
5.1.1 CASE OF OPERANT BOXES WITH LICK METER(S) 73
5.2 PACKWIN OPERANT MODULE (HO) 75
5.3 PACKWIN OPERANT MODULE (VT) 76

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5.4 PACKWIN FREEZING AND STARTLE MODULES (CSFR AND CSST) 77

6 SUBJECT DATABASE 78

6.1 SUBJECT MANAGEMENT 78
6.2 SUBJECT LISTS 80

7 EXPERIMENTAL PROTOCOL 81

7.1 THE PROTOCOL ORGANIZER 81

7.1.1 PROTOCOL TABLE 81
7.1.2 TOOL BAR 82
7.1.3 CREATE A NEW PROTOCOL 82
7.1.4 EDIT A PROTOCOL 84
7.1.5 DELETE A PROTOCOL 84
7.1.6 COPY A PROTOCOL 84
7.1.7 EXPORT AND IMPORT A PROTOCOL 85

7.2 EDIT A PROTOCOL WITH THE STATE EDITOR TOOL 86

7.2.1 PROTOCOL STRUCTURE 86
7.2.2 THE PROTOCOL INFORMATION PANEL 87
7.2.3 ENTER PROTOCOL GENERAL INFORMATION 88
7.2.4 CHOOSING A BOX TYPE 88
7.2.5 EDITING THE PROTOCOL 90
7.2.6 EDITING THE CHAIN OF STATE 93
7.2.7 THE STATE DATA SECTION 94
7.2.8 GLOBAL TRANSITIONS SECTION 107
7.2.9 EDIT A LIST OF STATES 109
7.2.10 EDIT A TIME OR EVENT LISTS 112
7.2.11 EDITING THE RUN-TIME PANELS 116
7.2.12 EXAMPLE OF FEAR CONDITIONING PROTOCOL 125
7.2.13 EXAMPLE OF STARTLE PROTOCOLS 128

7.3 EDIT A PROTOCOL WITH THE PACKWINHO ASSISTANT .. 132

7.3.1 SCIENTIFIC BACKGROUND 132
7.3.2 NINE HOLE PROTOCOL ASSISTANTS 134

7.4 EDIT A PROTOCOL WITH THE PACKWINVT ASSISTANT ... 144

7.4.1 SCIENTIFIC BACKGROUND 144
7.4.2 VOGEL TEST PROTOCOL ASSISTANTS 145

8 DATA ACQUISITION 153

8.1 CONDITIONS FOR ACQUISITION BUTTON AVAILABILITY 153

8.1.1 SYNCHRONIZED VIDEO RECORDING 153

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8.2 Associate Protocols and Boxes 154
8.3 Pairing Boxes and Cameras 155
8.4 Run-Time Panel & Data Acquisition Process 156
8.4.1 PACKWIN Operant Modules (CS, HO and VT) ..156
8.4.2 PACKWIN Freezing and Startle Module (CSFR and CSST) 165
8.5 Starting and stopping acquisition with the remote control 185
8.5.1 PACKWIN Operant Module 185
8.5.2 PACKWIN Freezing and Startle Module (CSFR and CSST) 186
9 DATA ANALYSIS 187
9.1 The Analysis main window 187
9.1.1 Session Summary Table 187
9.1.2 Session Info 188
9.2 Filter the sessions to analyze 189
9.3 Select the sessions to analyze 190
9.4 Generate a single-session analysis report 191
9.4.1 PACKWIN Operant Modules (CS, HO and VT) ..191
9.4.2 PACKWIN Freezing Module (CSFR) 218
9.4.3 PACKWIN Startle Module (CSST) 225
9.5 Generate a Multi-session analysis report (batch analysis) 230
9.5.1 PACKWIN Operant Module (CS) 230
9.5.2 PACKWIN Operant Nine Hole Module (HO) 248
9.5.3 PACKWIN Operant Vogel Test Module (VT) 252
9.5.4 PACKWIN Freezing Module (CSFR) 259
9.5.5 PACKWIN Startle Module (CSST) 266
10 EXPERIMENTAL FILES MANAGEMENT 272
10.1 Structure of the experimental file 272
10.2 Changing the localization 272
10.3 Changing file name 272
10.4 Duplicating an experimental file 273
10.5 Size limitations 273
11 PACKWINCS EXTENSION 274
11.1 Combining Assistant tools and the State editor tool 274
11.2 Editing the Run-Time panel 275
11.3 Editing a customized report 275

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12 RECORD-IT! MEDIA INTEGRATION 276
12.1 RECORD-IT! MEDIA INSTALLATION 276
12.2 VIDEO RECORDING SETTINGS 276
13 CONTACT INFORMATION 278
PANLAB 278
TECHNICAL SUPPORT 278

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1 INTRODUCTION

The establishment of a software program for controlling a behavioural experiment is always a complex task. Panlab proposes PACKWIN, whose user-friendliness and versatility offer a powerful tool to develop a wide range of experiments in different types of behaviour chambers.

PACKWIN typically controls the Panlab chamber for Operant and Non-Operant Conditioning (Startle Reflex / Fear Conditioning), Self-Administration and procedures using the Nine-Hole box, but it can also monitor any type of standard input/output operant chambers.

This manual contains material for the experimenter who wishes to use PACKWIN for design of behavioural tests, their measurement, and analysis.

We recommend the user to carefully read the whole manual before beginning an experiment to avoid any hardware damage and software malfunctioning due to any possible misuse of the system.

As required in the Good Laboratory Practices (GLP) directives and instruction, PACKWIN has been built in order to obtain traceable data: i.e. each session recorded can be linked to the corresponding experimental data (date, experimenter, animal data, protocol used, etc.).

At any moment, the user can request support of the Panlab Scientific Department for any personalized help for the configuration and the use of the PACKWIN software.

1.1 What's New in PACKWIN 2.0?

- New modular structure: save money and gain convenience by only purchasing what you need!!!!
- Wider range of experiments: Integration of Operant and Non-Operant Conditioning (Startle Reflex and Fear Conditioning) experiments in a single software package.
- Even more user-friendly interface! New Launch Assistant and new Experiment Assistant bar for guiding the user through the different steps of the experiment.
- Fully integrated with RECORD-IT! Media.
- New Box Test option for checking the correct working of all the input and output elements of the box directly from the software (and without having to edit or use a specific "Test element" experiment file).
- Updated Subject & Group Database structure, convenient for the experimenter.

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- New assistant panel and specific reports for standard 5-9 holes experiments (5-Serial Choice Reaction Task procedure) and Vogel test. Straightforward protocol configuration and nine-hole targeted reports.
- New module for Fear conditioning and Startle reflex experiment (integration of the FREEZING and STARTLE software of the STARTFEAR system).
- New simulator option for creating and checking protocols even when the boxes are not connected (at your desk or at home)!
- Easy Importation & Exportation of protocols between the different experiment files.
- Easy Importation & Exportation of the event and time lists between different protocols or between different experiment files.
- New global transition option in the protocol editor for indicating from which state the global transition has to be taken into account.
- New capability for an automated monitoring of yoked procedures.
- Batch analysis and direct exportation to Excel (1 row per subject reports).
- New time selector for changing the START/STOP interval of analysis of the session. Chose the portion of the session to be analysed!
- New analysis data report by user-defined intervals of time
- New integrated response pattern plots for group analysis.
- Use of PACKWIN software not exclusive to Panlab chambers! Can be used with chambers from other brand provided with standard Input/output elements.
- Software deployment through USB flash key. Direct free trial download from Internet – Shorter delivery time – Easy access to updates and upgrades.
- Panlab InTouch! A new innovative communication system to be in touch with customers and to keep them up to date with news, events, special offers, scientific publications and relevant information related to the Panlab products acquired. With Panlab In-Touch! customers can also reach Panlab to get tech support, give their opinion about the software, new products or features or just let Panlab know any idea to improve the experience.

1.2 A new modular structure

From the version 2.0, PACKWIN is proposed with a new modular structure composed of a platform, PACKWIN 2.0, and specific experiment modules. All the experiment modules available can be used independently and can be classified in 3 main experiment groups:

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- **OPERANT BEHAVIOR module**
    - ***PACKWIN-CS:*** Customized operant procedures modules

      Flexible and advanced module providing specific panel tools to configure basic schedules for operant procedure, more specific and complex user-defined protocols, as well as a wide variety of data reports completely configurable by the user.
    - ***PACKWIN-HO:*** 5/9 hole module

      Platform providing an easy configuration assistant, predefined Run-Time panel, and specific data report for experiments related to the 5-Choice Serial Reaction Task (5-CSRT) test for visual attention.
    - ***PACKWIN-VT***: Vogel test module

      Platform providing an easy configuration assistant, predefined Run-Time panel, and specific data report for experiments related to Vogel test for the screening of anxiolytic properties of drugs.

- **FREEZING module**
    - ***PACKWIN-CSFR***: Customized Freezing module

      Flexible and advanced module providing specific panel tools allowing the configuration of basic schedules for Non-Operant Fear Conditioning procedures as well as data reports completely configurable by the user.

- **STARTLE module**
    - ***PACKWIN-CSST***: Customized Startle module

      Flexible and advanced module providing specific panel tools allowing the configuration of basic schedules for Non-Operant Startle Reflex procedures as well as data reports completely configurable by the user.

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	OPERANT PROCEDURES	FREEZING	STARTLE
Customized modules *(free edition of protocols and analysis reports)*	PACKWIN-CS	PACKWIN-CSFR	PACKWIN-CSST
Experiment-targeted modules *(specific protocol asistants and pre-configured analysis reports)*	PACKWIN-HO PACKWIN-VT	*Not yet available!*	*Not yet available!*

The PACKWIN 2.0 platform provides a shared structure/function for most of the steps of the experimental process (Box definition, Box test, Subject Database, Protocols Database, Data Acquisition and Data Analysis), but also some specificities (specific installation procedures, specific box elements, specific protocols configurations, specific Run-Time panel and analysis report capabilities).

The PACKWIN-HO and VT modules can be extended to the PACKWIN-CS module for adding advanced protocols and analysis configurations functions to the experiments.

The PACKWIN-CS module can be extended to the PACKWIN-HO and VT modules for getting straightforward standard protocol assistant and time-saving preconfigured analysis reports.

1.3 System Packaging

The complete system includes experimental boxes, control units, and PACKWIN software accompanied with their cables and respective User's Manual.

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1.3.1 Experimental Boxes and Accessories

PACKWIN can control a wide range of compatible experimental boxes (behavioral chambers) depending on the PACKWIN module used.

PACKWIN MODULES	EXPERIMENTAL BOXES COMPATIBILITY
PACKWINCS	Panlab compact and modular operant boxes Panlab 5/9 holes boxes Panlab Vogel test boxes Panlab Shuttle boxes Possibility to adapt to other brands (Med Associates, Coulbourn...), contact us for more information.
PACKWINHO	Panlab 5/9 holes boxes
PACKWINVT	Panlab Vogel test boxes
PACKWINCSFR	Panlab Fear conditioning boxes
PACKWINCSST	Panlab Startle boxes

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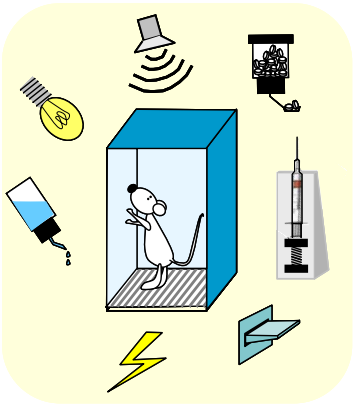
1.3.1.1 PACKWIN OPERANT MODULES (CS, HO and VT)

PACKWIN CS

PACKWINCS can control operant boxes provided that the elements are standard input/output elements.



Basically, the experimental boxes are defined by a chamber and some elements (light, sound, lever, pump, food or drink dispenser, etc.).

The elements allow the animal to interact with the experimental paradigm defined in the PACKWIN protocol editor.

Panlab operant boxes can be completely modular (user-defined elements) or compact (predefined elements).

Example of modular operant boxes: classical operant boxes (Skinner boxes, self-administration boxes, intracranial stimulation boxes, Geller-Seifter boxes etc...

Example of compact operant boxes: 5/9 holes boxes, Vogel test boxes, shuttle boxes...

In modular operant boxes, the user defines during the purchase process the elements to be included in the box. Additional elements can also be purchased and installed afterwards depending on the experiments planned by the user.

In any case, all the changes planned by the user have to be made under the supervision of the provider.

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Several accessories are available depending on the box configuration purchased (shockers, injection pump controller…). See the Hardware User's Manual for more details about the use and technical specifications for these devices.

PACKWIN HO

The Nine-hole module is related to the Nine-hole box configurations provided by Panlab: the 5/9 hole box (LE509MO and LE507MO).

The standard **Nine Hole boxes** for rat and mice are provided with the following elements:

- 9 Holes with individual led and photocell detectors
- 1 Pellet dispenser with photocell detectors
- 1 House light
- 1 Manual button

PACKWIN VT

The Vogel test module is related to two specific Vogel test box configurations provides by Panlab, the Vogel test box for rat and mouse (LE862 and LE865).

The experimental box by it-self is a standard home cage with lid, a bottle, a lickometer spout, and shocker connections.

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The Vogel box is provided with the following elements:

- 1 lickometer
- 1 shock dispenser

The Vogel Test module of PACKWIN can also be used with any Panlab operant box provided with**, at least,** the following elements:

- 1 lickometer
- 1 shock dispenser
- 1 House light

A shocker is used as additional accessory to the system to deliver shocks when the subject has made a defined number of licks. See the Hardware User's Manual for more details about the use and technical specifications for this device.

1.3.1.2 PACKWIN FREEZING AND STARTLE MODULES (CSFR and CSST)

The FREEZING and STARTLE experimental boxes are defined by a box and specific available elements such as Tone, Prepulse/Pulse sound, light, air puff and shock. In this box, the animal activity/freezing and startle reflex is detected through the use of weight transducers placed under the floor.

The elements of the box allow the animal interacting with the experimental paradigm defined in the PACKWIN protocol editors.

The boxes also need the following accessories:

- LE111 LOAD CELL control unit

The LE111 control unit allows the calibration and the amplification of the signal registered through the weight transducers placed under the floor of the box. One LE111 control unit is required for each STARTLE / FREEZING experimental box.

- LE100-26 SHOCKER (optional)

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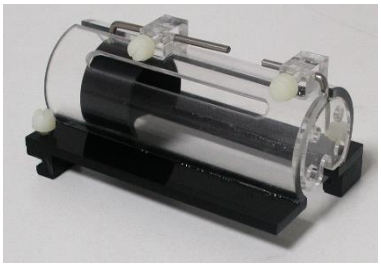
The LE100-26 shocker unit controls the intensity of the shock stimuli provided during the experiment. This accessory is used for fear conditioning studies and fear-potentiated startle reflex experiments. One LE100-26 shocker is required for each STARTLE experimental box.

- LE117 series Holders



The LE117 series of holders are only used during startle reflex experiment to minimize the animal movement during the test. The size holder has to be large enough to minimize the stress induced by the containment, but small enough so the animal does not easily move around. An animal holder habituation phase is recommended.

- LE115 Contextual kit

The contextual kit is used in cue-dependent fear conditioning experiments for changing the context during the test phase of the procedure.

1.3.2 Interface Units

These devices interface between the experimental boxes, accessories, and the computer where PACKWIN will be installed.

The interface units are different depending on the PACKWIN experimental modules used. OPERANT modules use LINKBOX-type interfaces, STARTLE/FREEZING modules use LE118-type interfaces.

1.3.2.1 PACKWIN OPERANT MODULES (CS, HO and VT)

The Linkbox is an "input/output" (I/O) interface between the operant box (with the elements included) and the computer.

The Linkbox is used as a gathering system for the different elements of the experimental box. Each element will be connected to a given I/O using a TTL-TTL cable.

The exact location of each connection is given by the provider and can only be changed by the user under the supervision of the provider.

The Linkbox for classical operant boxes (skinner box, self-administration box, Vogel test box...) is provided with 8 inputs and 16 outputs:

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The latest version of the Linkbox is the LINBOX01HS (High-speed). PACKWIN 2.0.09 is compatible with both versions.

The link box for the standard **Nine Hole box** for rat and mice is provided with 10 inputs and 11 outputs:

The latest version of the Nine Hole box is the Nine Hole box HS (High-speed). PACKWIN 2.0.09 is compatible with both versions.

1.3.2.2 PACKWIN FREEZING AND STARTLE MODULES (CSFR AND CSST)

The LE118 control unit is used as a stimulus interface between the STARTLE experimental boxes and the computer.

The LE118-8 control unit is used to connect up to 8 STARTLE experimental boxes to the computer.

1.3.3 Computer

The computer used with PACKWIN can be either a desktop PC or a laptop PC. For FREEZING and STARTLE experiments a desktop PC is recommended due to the

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sound card requirements. See the Installation chapter for more details about the computer requirements associated to each specific module.

1.3.4 The PACKWIN Software & USB Key

- PACKWIN software package contains:
    - A USB flash key (with software installer, License rights, and digital User's Manual)
    - A START/STOP remote control for data acquisition
    - A RS232/USB adapter (only needed for the connection to the boxes working with the OPERANT module!).

PACKWIN software and the modules licensed are delivered within a single USB flash key. The key is named PBLICENSE and contains:

- the software installation tool
- the software User's Manual in PDF format
- specific components required to work
- module License management

Due to security reasons of the Windows® operating system, a user with administrative rights is required to install the software and other components. Please contact your IT staff before installing the software.

Please **DO NOT rename** the USB title (its name should always be PBLICENSE).
Please **DO NOT remove or modify any file** stored within the USB flash key, especially the regkey.dat file which stores the information regarding your license of use.

The software can be installed on any computer as required.

The PACKWIN USB key is only needed for the following operations:

- software installation
- data acquisition
- software update and upgrade

Protocol configuration and data analysis can be performed on as many computers as required by the user without having the USB key connected to the computer (except for the first software installation and for software update/upgrade processes).

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2 SYSTEM INSTALLATION

The installation of the whole system is divided in the following steps:

1. Hardware connection
2. Software installation
3. System calibration & checking

See the Hardware user manual for the step 1. Steps 2 and 3 will be described in the following chapters with the differences explained for each experiment module.

2.1 Computer Requirements

Computer requirements for PACKWIN software.

- A fully compatible computer with at least:
  - Intel Core™ i7-10700 (10th gen) processor or superior (Celeron processor not supported).
  - 8 GB of RAM or superior.
  - HD 250 Gb (150 MB of free hard disk space).
  - Graphics: 1024x768 pixels and 32-bit true colour.
- Connection interface

  - One free USB port for the software USB flash key.
  - One free USB port for the box connection.

  Note: when using PACKWIN with operant boxes a RS232(serial) /USB adapter is included in the software package. The FREEZING and STARTLE modules are provided with direct USB communication (no adapter is needed in this case).
- Sound card (only for FREEZING and STARTLE modules)
  - The tone used by the PACKWIN-CSFR and PACKWIN-CSST module use the computer sound card. In that case, using desktop is recommended instead of laptop. The PCI sound card must measure RMS at least at 0,5 Volts (see chapter 4.1.4.2. for details about how to test the computer sound card).
- Operating system supported:
  - Microsoft® Windows® 11 64 bits.
  - Microsoft® Windows® 10 32 and 64 bits.
- External Software needed:
  - Microsoft Excel ®

If the external software is not available, some analysis reports could not be generated due to an "Excel/Word OLE Server not found" error. Please contact your IT staff in order to install the external software before analysing sessions.

- Printer (recommended).

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- Computer administrator rights

  Please check that your user has administrative rights on the PC or laptop in which the software or device is to be installed. Please contact your IT staff in order to clarify this issue before the installation procedure will be done.

2.2 Box Assembly & Connection to the Interfaces

2.2.1 PACKWIN Operant Modules (CS, HO and VT)

Please refer to the hardware User's Manual for the details about how to assemble the different operant hardware elements (response modules, stimuli, reinforcements…) and accessories connected to the experimental boxes.

To connect the elements of the operant boxes to the corresponding Linkbox please follow the instructions of the document "Schema of connections" provided with the PACKWIN software.

During the connection process, the link box has to be always switched off.

To do that:

- Connect the PACKWIN USB key to a computer
- Double-click on the **Panlab.exe** file
- Click on the **Schema of connections (Operant)** to open a pdf file with the instructions to connect the different box elements of your system to the link boxes

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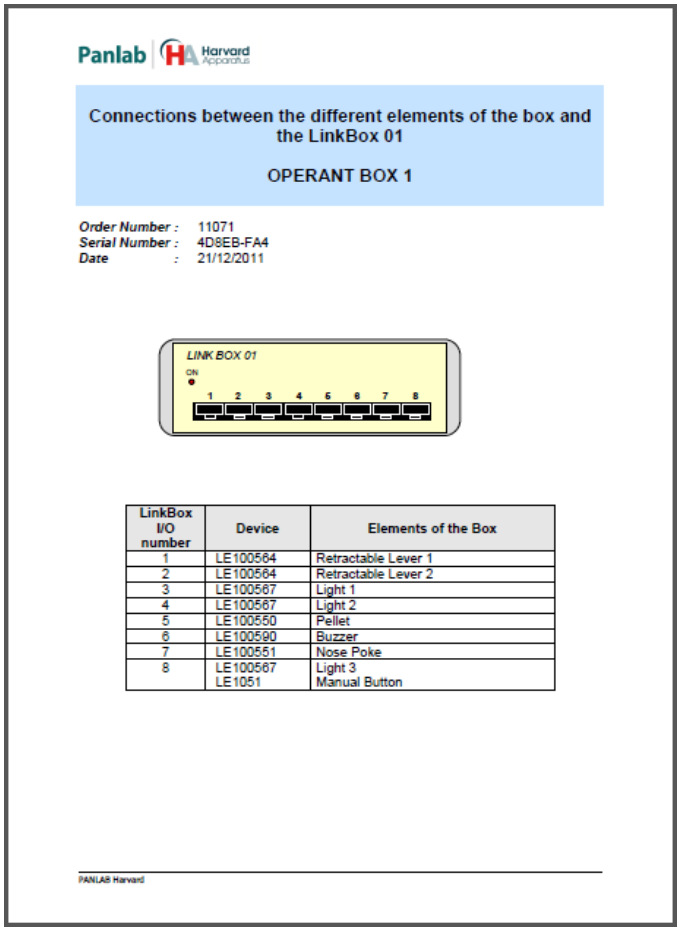

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Connections between the different elements of the box and the LinkBox 01

OPERANT BOX 1

Order Number : 11071
Serial Number : 4D8EB-FA4
Date : 21/12/2011

LinkBox I/O number	Device	Elements of the Box
1	LE100564	Retractable Lever 1
2	LE100564	Retractable Lever 2
3	LE100567	Light 1
4	LE100567	Light 2
5	LE100550	Pellet
6	LE100590	Buzzer
7	LE100551	Nose Poke
8	LE100567 LE1051	Light 3 Manual Button

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In order to avoid unpredictable behaviors by PACKWIN, it is mandatory that the connections respect the location indicated by the provider in the specific Schema of connection document.

2.2.2 PACKWIN Freezing and Startle Module (CSFR and CSST)

Please refer to the hardware User's Manual for the details about how to connect the experimental boxes to the different accessories (LE111 load cell, LE100-26 shocker, LE117 holder, LE119 air puff etc.) and how to connect the different LE111 units to the LE118 or LE118-8 interfaces.

2.3 Set the Link Box Identification Number (Only for Operant Modules)

2.3.1 PACKWIN Operant Modules (CS, HO and VT)

When several boxes (*n* boxes) are used, a different ID number has to be set by the user on the rear panel of each corresponding Linkbox (consecutive numbers from 1 to *n*).

In the 5/9 Hole box-type Linkbox, the ID numbers are set internally by the manufacturer and each Linkbox is labelled accordingly.

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2.3.2 PACKWIN Freezing & Startle Module (CSFR and CSST)

The box system associated with the PACKWIN FREEZING and STARTLE modules uses a unique interface (LE118-8) for up to 8 boxes. Setting an identification number for the LE118 interface is not needed.

2.4 Install USB Connection Drivers

2.4.1 PACKWIN Operant Modules (CS, HO and VT)

If more than 1 chamber is used, the Linkbox are connected sequentially from the first to the last one using the specific RS232 cable provided with the system.

The first Linkbox of the chain is connected to the computer using the RS232/USB adapter provided with the software.

2.4.1.1 CONRS232USB-HS converter (high speed mode)

The use of PACKWIN with the Linkbox01 HS (High Speed) requires the use of the CONRS232USB-HS converter (USB2-H1002).

WARNING: do not use direct connection between the Linkbox and the computer RS232 serial port (if any).

The RS232/USB converter is needed for converting a USB port to a serial port valid for communications between hardware and software. It allows the use of two serial ports in your PC or laptop. We recommend the use of a specific model of converter. We cannot guarantee the correct functioning of the system with any other USB-serial converter. The converter includes an extension cable just in case.

To Install the converter:

1. Connect the converter to the computer.
2. Windows will automatically install the drivers.
3. If working with a Windows 7 or previous, please refer to the notice provided in the box of the converter.

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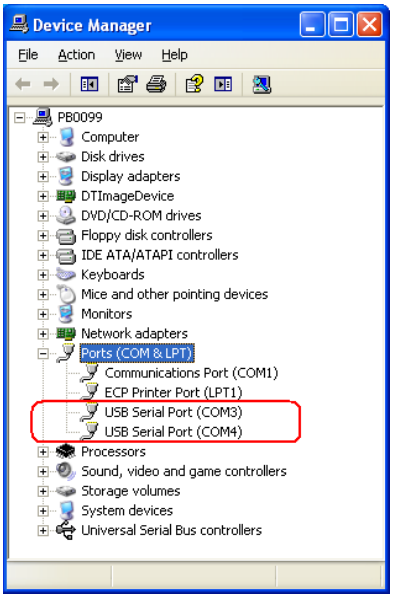
4. Once connected and installed, two serial ports will appear into the [Device Manager] window on the Windows Operative System. Usually, the numbers assigned by Windows are sequential.



2.4.1.2 CONRS232USB (blue) converter (legacy mode)

The blue RS232/converter was included in PACKWIN V2.0.06 (and older) software package. This converter does not allow the system to work in the high speed mode, even if you are connecting a Linkbox with high speed capacities.

PACKWIN 2.0.09 is still compatible with the use of the system in a Legacy mode (not high-speed). In case you need to re-install this device, please follow the below procedure:

You need to have administrator privileges to install any new drivers. Please contact your IT staff in order to clarify this issue before continuing installation of the device.

- The drivers should be installed prior to hardware installation. Do not connect the blue adapter to the USB port of your computer before you finish driver installation.
- Insert the PACKWIN software USB flash key into a free USB port of your computer, access its content, and execute the installation assistant (Panlab.exe).
- The following installation window will be shown. Press the [Install Drivers USB-RS232] option to start the software installation process.

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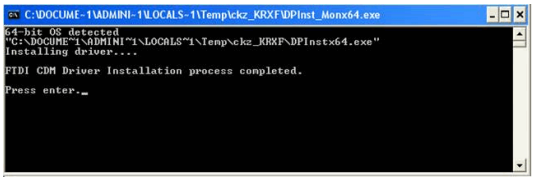
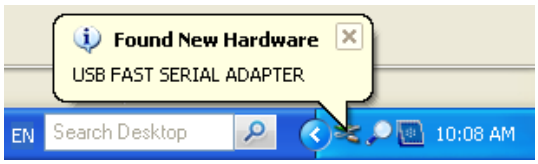
- The USB COM installation program will auto-detect the OS type and install the driver automatically. In some operating systems a dialog box may appear asking to press [ENTER] at the end of the installation.



- After the message "FTDI CDM Driver installation process completed" appears, press [Enter] to complete the driver installation.
- Plug the blue adapter in any USB port of your computer. Windows will finish installing the driver files.

- In the lower right corner of the screen the next message will be automatically shown:



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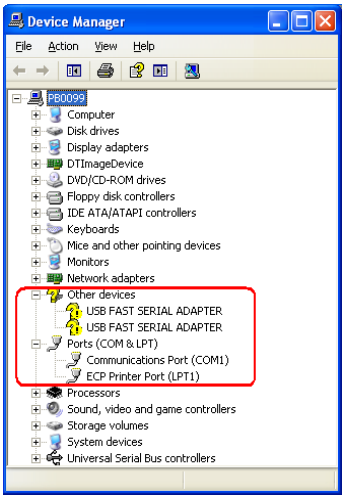
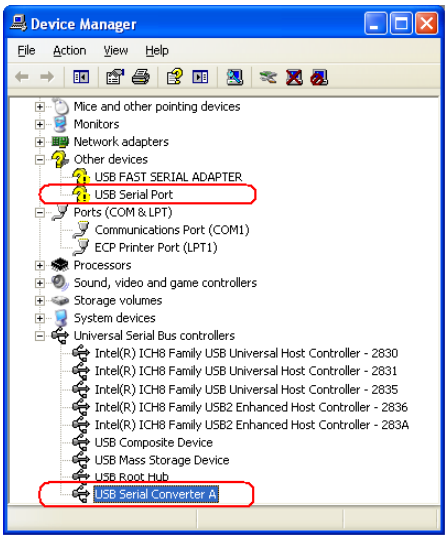
- At the same time, two devices will appear into the [Device Manager] window. The ports provided by the new [USB FAST SERIAL ADAPTER] will be shown under [Other devices] with a warning sign attached.



- Please, wait while the wizard locates the drivers installed previously. This process may require several minutes depending on your PC.
- The process of the correct activation of the device (that is, when the PC or laptop recognizes the new serial port), is done one by one.



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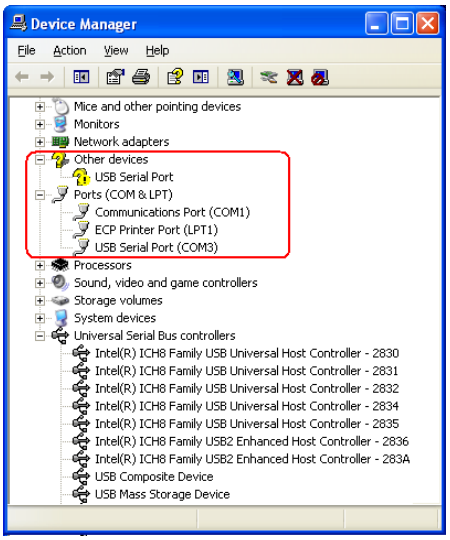
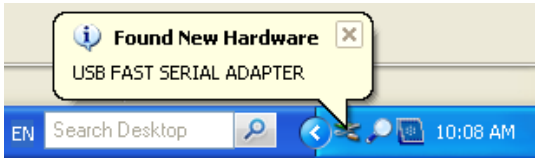
- The next picture shows how the number of the port is finally assigned by the system.



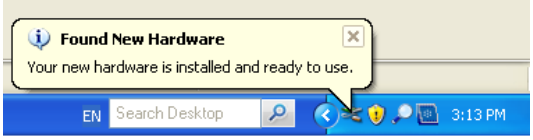
- When the wizard finishes, it will ask you to press the [FINISH] button.

Important remark:

Until now, only one serial port has been correctly installed. The process must be repeated for the second port. Please, wait while your PC or laptop finds another COM port. Once again, the next message will appear in the lower corner of the screen:



- The adapter will be correctly installed when all previous steps have been repeated. Finally, the message will appear in the lower right corner of the screen.



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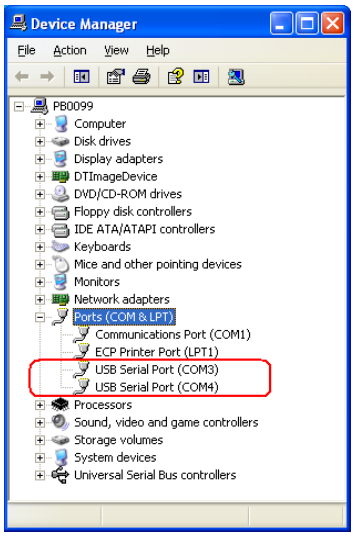
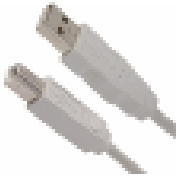
- At the same time, the two serial ports will appear into the [Device Manager] window. Usually, the numbers assigned by the system are sequential.



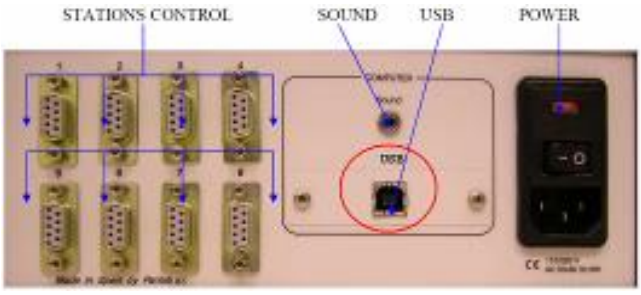
A yellow label with the text [Port 1] is attached to the adapter device to identify the first port recognized for the computer system. That means that if [Device Manager] shows two ports (COM3 and COM4), then that label [Port 1] corresponds to COM3.

2.4.2 PACKWIN Freezing & Startle Module (CSFR and CSST)

The more recent version of the FREEZING and STARTLE systems uses direct USB connection between the LE118 and the computer. Specific USB drivers have to be installed in the computer for allowing the detection of the LE118 box interface by the software installed on the computer.



- Before installing the drivers in the computer all the applications must be closed and the device **LE-118 must be unplugged**.



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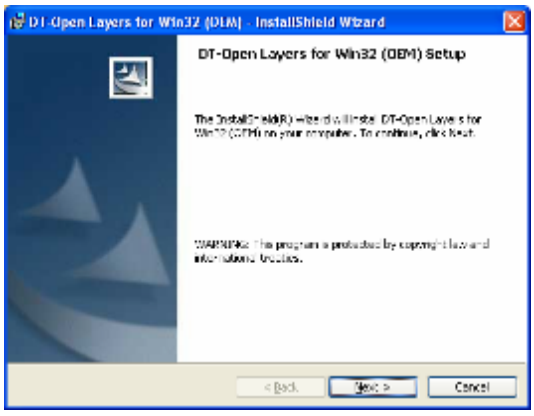
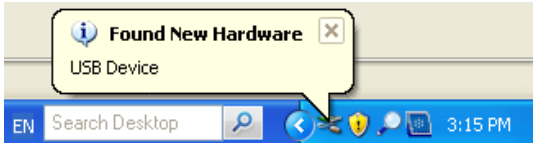
- Plug the USB flash key in a free USB port of your computer and execute the PANLAB.EXE file.

- Click on the "Install USB Device Drivers (Startle/Freezing)" option.

- An installation wizard will be automatically shown. When the Welcome screen appears, click [Next] and [Install] button to continue. Wait until the wizard asks for you to press the [FINISH] button.



- Once done, the LE118-8 device must be plugged in and in the lower right corner of the screen will appear the next message.



- The wizard for installing the drivers for the [Found New Hardware] will run. Select the option [Yes, this time only] and press the [Next] button to continue. In the next window choose [Install the software automatically] and press the [NEXT] button to recognize the LE118 device.

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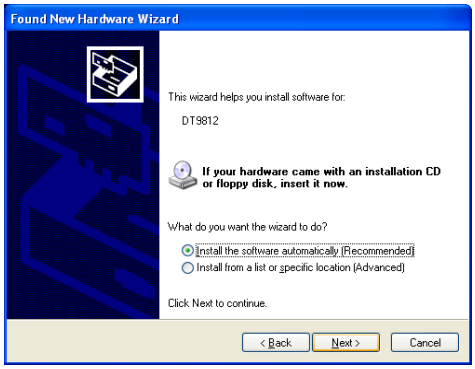
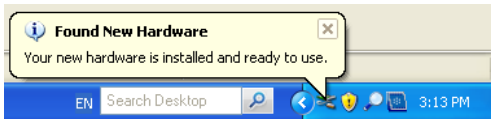
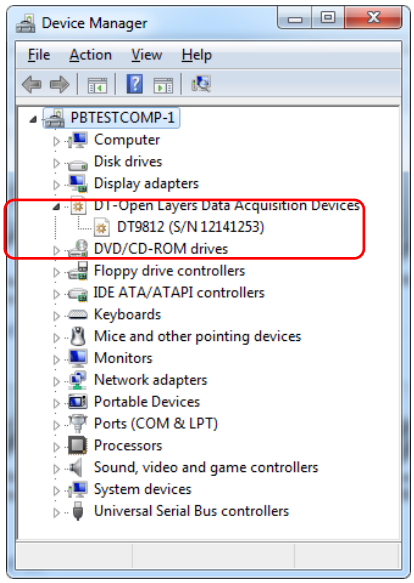


- Please, wait while the wizard searches the drivers installed previously. This process will need some minutes depending on your PC. When the wizard finishes will ask you for press the [FINISH] button.

- In the lower right corner of the screen will appear the next message:



To verify the right installation of the drivers, access the [Device Manager] option of the PC as is shown in the next picture:



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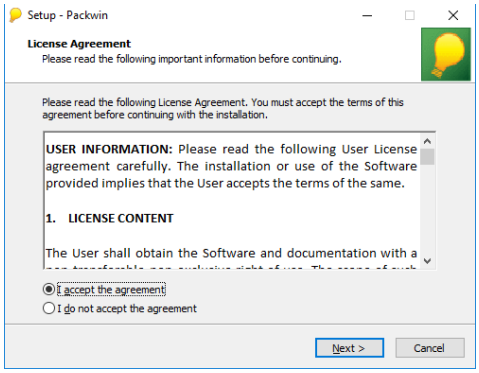
2.5 Install the PACKWIN Software

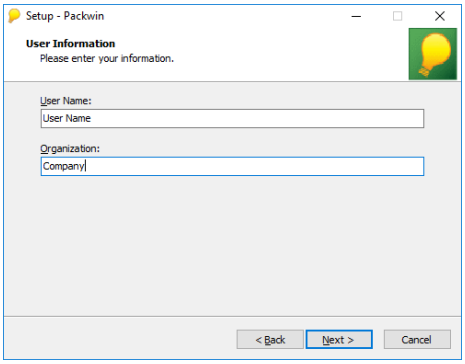
The next steps have to be followed for installing the software on the computer that will be used for the experiments:

- Connect the PACKWIN USB key to a computer
- Double-click on the **Panlab.exe** file
- Click on **Install PACKWIN v2.0.09** for initiating the software installation process

- An installation wizard will appear. Read carefully the **License Agreement** statement and check **the "I accept the terms of the license agreement"** option to continue the installation of PACKWIN. Then press the [Next] button to start the installation.



- In the next window, enter the name of the user and the company in the corresponding fields. Press [Next] button to continue.



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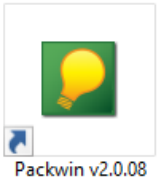

- Press the [Next] button until reaching the [Finish] button.

A new shortcut will appear on your desktop. Use it for executing the program later. If the **Run PACKWIN v2.0.09** now box was checked in the last installation window, then PACKWIN will be automatically launched at the end of the software installation process.

2.5.1 The USB protection key management

A software protection key is a device used to prevent software from unlawful use. This small part is connected to the computer via a free USB 2.0 port. Only one key is provided per purchased license, so it is not possible to record data on more than one computer at a time.

2.5.1.1 Data Acquisition

Data acquisition (session registering) from connected hardware can only be performed when the USB flash license key is plugged in to the computer running the PACKWIN software.

Please DO NOT remove or modify any file stored within the USB flash key, especially the regkey.dat file which stores the information regarding your license of use.

To make use of the protection key, make sure it is plugged in before PACKWIN is started. If no protection key is present, data acquisition will not be possible.

2.5.1.2 PROTOCOL EDITING AND DATA ANALYSIS

Protocol configuration, protocol validation, and data analysis (data report generation) does not require the license key and can be performed on as many computers as needed by the user.

To install PACKWIN on another computer and use it for all of these functions, the following installation steps need to be followed:

- The USB flash license key must be plugged in to the computer.
- The PACKWIN software should be installed and should be executed at least one time.
- To check the correct installation of the software for analysis, please go to the Help - About panel and check the information provided in the SERIAL NUMBER and MODULE sections.

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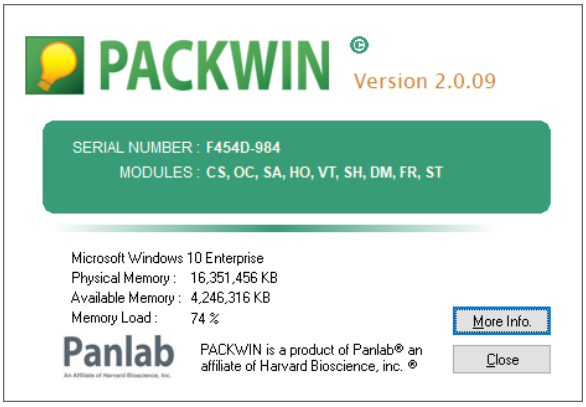


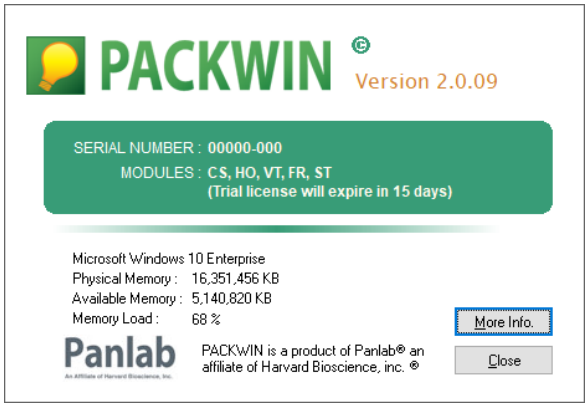
- From that point, PACKWIN will no longer require the USB flash key in order to edit protocols and analyze data.
-

2.5.2 Evaluating PACKWIN: the trial period

This section only applies if you have acquired a trial version of PACKWIN available for download at http://www.panlab.com/.

The trial period is of **15 days** counted from the first time PACKWIN is executed and is intended for testing purposes. During this period, PACKWIN will be fully functional. This means that every module and every extension will be available.

The trial period information can be accessed through the Help – About menu option:



The "Trial Version" field shows the status of the trial period.

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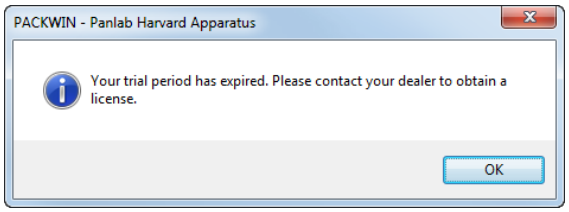
A week (7 days) before the trial's expiration date, PACKWIN will show a warning that the trial period will expire, with instructions on how to purchase your licensed edition of the software.

If the trial period is definitely expired, a warning message is shown and PACKWIN will no longer execute.



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2.6 Install the Configuration File

2.6.1 PACKWIN Operant Modules (CS, HO and VT)

PACKWIN comes with a trial version of the box configuration file with a demo set of box definitions (demo jaulas.box and LinkboxRS232.cfg files) that can be used for learning more about the system before the purchase process.

Once the customer purchases their own boxes, their specific box configuration files (Jaulas.box and LinkboxRS232.cfg) provided in the PACKWIN USB key have to be installed. Both files **must be copied manually** to the same folder in which PACKWIN is installed.

To do that, follow the next instructions:

- Plug the USB flash key in a free USB port of your computer.
- Execute the **PANLAB.EXE** file.
- Click on the **Box configuration file** option.

- Copy the **Jaulas.box** and **LinkboxRS232.cfg** files from the current folder to the PACKWIN's installation folder. The default PACKWIN's installation folder is located in "[Drive]:\Program Files\Panlab\PACKWIN v2.0".

During the use of the system, the box configuration files play a key role and should not be eliminated or manipulated by the user. They allow the PACKWIN software to identify the chambers that will be used during the experiment as well as the characteristics of the different modules plugged to each chamber.

The **jaulas.box** file has the following functions:

- providing PACKWIN with the list of the elements connected to the experimental boxes.
- providing PACKWIN with the information about the I/O entry number in the link box (number 1 to 8) in which each element has been connected.
- Providing PACKWIN the information about the functional properties of each element.

2.6.2 PACKWIN Freezing and Startle Modules (CSFR and CSST)

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The configuration file provided with the PACKWIN software for the fear conditioning and startle box are common to any users and are installed automatically during the installation of the PACKWIN software. No additional operation has to be done by the user.

2.7 Experimental File Example

2.7.1 PACKWIN Operant Modules (CS, HO and VT)

The **Box configuration files (operant)** option also gives access to ready-to-use experiment files according to specific needs. Such examples of experimental file are not provided with any of the PACKWIN configurations. Please contact your dealer if you need support on this point.

2.7.2 PACKWIN Freezing Module (CSFR)

Some experimental file examples are provided with the PACKWIN software.

- Fear conditioning test elements experiment files
- Fear conditioning example experiment files

These files are installed directly into the PACKWIN **Experiments** folder created by the application during the PACKWIN software installation.

2.7.3 PACKWIN Startle Module (CSST)

Some experimental file examples are provided with the PACKWIN software.

- Startle test elements experiment files
- Startle examples experiment files

These files are installed directly into the PACKWIN **Experiments** folder created by the application during the PACKWIN software installation.

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2.8 Install the Remote Control

Teleswitch unit allows for remote control using the radiofrequency technique for controlling the start and stop of the session without the computer. It is especially useful when the experimental protocol requires the data acquisition process to start at the same time the subject is placed into the experimental area. The remote start of the session in this context can be then achieved using the teleswitch unit.

2.8.1 Connecting the Teleswitch

The teleswitch unit is composed of a remote-control unit with a small USB wireless adapter device.

Before connecting the teleswitch unit to the computer, make sure to install the battery (included) in the back of the unit:

Version 1	Version 2

Now remove the USB receiver from the back of the unit and plug it into a free USB 2.0 port in your computer.

Version 1	Version 2

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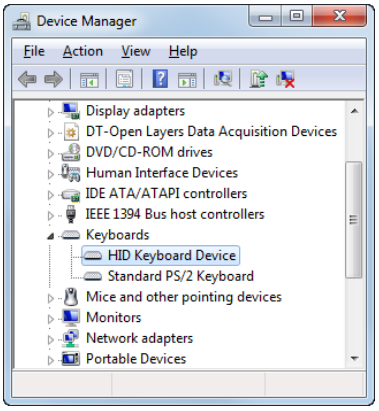
The recommended distance between the teleswitch unit and the USB receiver plugged into the computer is 10 meters without any obstacle.

An additional USB extension cable is provided to facilitate installing the USB receiver in a position in which the visibility is improved.

If needed, plug one side of the USB extension cable into the free USB 2.0 port in your computer and the USB receiver into the other side of the cable. Then put the USB receiver in a stable position without any obstacle between it and the teleswitch unit.

Now wait for Microsoft® Windows® to automatically detect and install the new device.

In order to check if the system has recognized and installed, the new device accesses the "Device Manager" on the Control Panel and check under the key "Keyboards" that a new entry "HDI Keyboard Device" has been added.



2.8.2 Starting and Stopping Trials with The Teleswitch

Data acquisition sessions (trials) can be started and stopped by means of the corresponding buttons of the teleswitch unit as indicated:

Version 1	Version 2

2.9 Additional Computer Configurations

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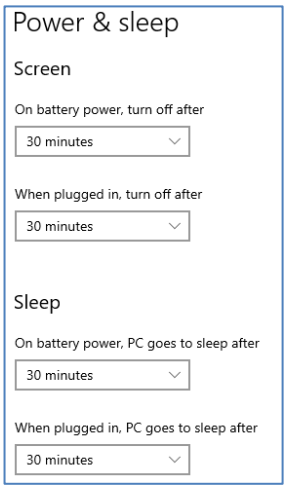
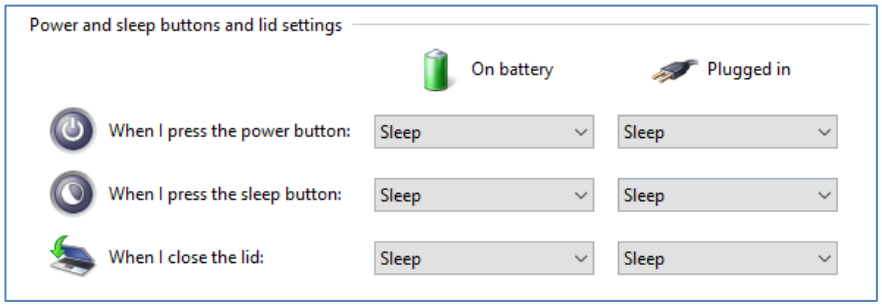
It is important to always set the Power Energy settings accordingly to the length of the experiment that will be performed to avoid a Sleep or Hibernation during the Data Acquisition.





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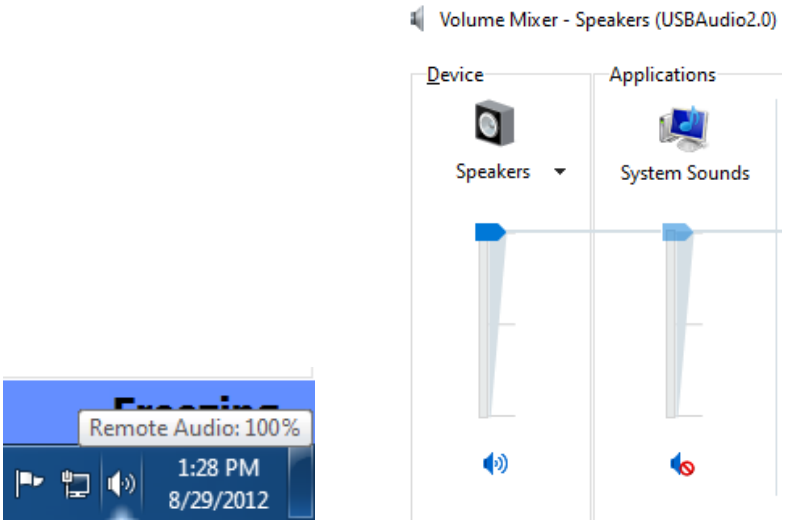
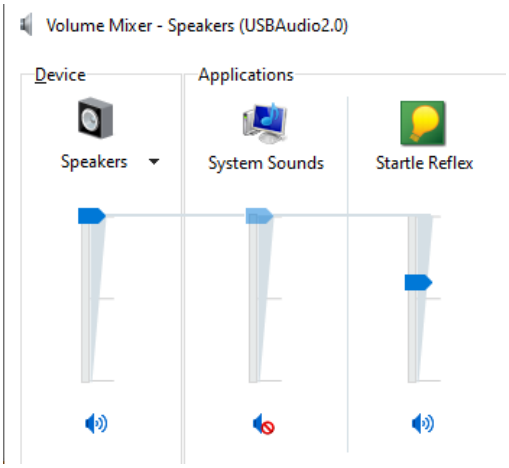
When using the FREEZING and STARTLE modules, sound frequencies and intensities are controlled by the software using the PC-integrated sound card. The PC sound output has to be then configured to fit with the FREEZING and STARTLE experimental requirements.

2.9.1 Automatic Setting to Maximum Volume

When an experiment of the FREEZING and STARTLE module is initiated, the sound ***Volume*** option of the PC is automatically set to maximum to ensure the maximal potency of the sounds given during the experiment. The ***Mute*** option has to be manually activated for System Sounds.



When detecting the box in PACKWIN (see chapter 4.1.4), the volume of PACKWIN is also automatically set to a default value to ensure an optimal stability of the output sound signal.



This process allows all the computers used in the FREEZING and STARTLE boxes to have the same sound characteristics.

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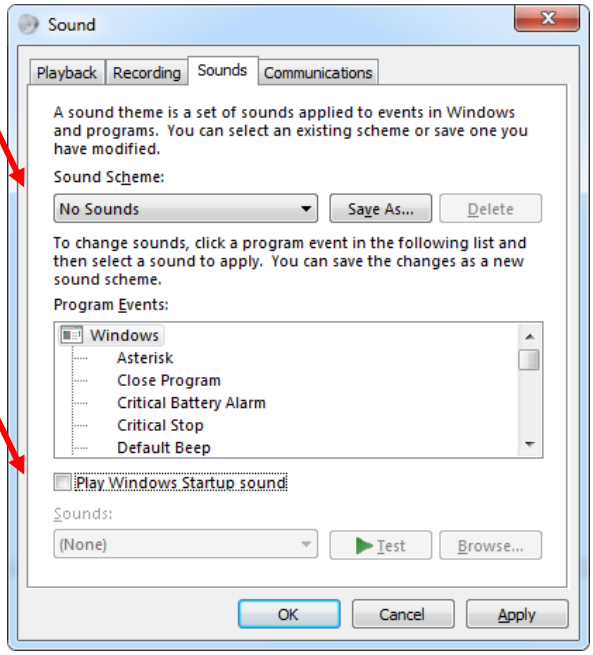
2.9.2 Sounds Scheme Deactivation

By default, the sound generated by the card is combined with some events in Windows and the programs used: program opening, alarms etc... These combinations have to be muted by selecting the "***No Sounds***" option and unchecking the "***Play Windows Startup sound***" option in the ***Sound*** panel (Start \ Control Panel \ Hardware and Sound \ Sound \ Change system sounds) and press the [Accept] button (if desired, the system gives the user the possibility to save the previous sounds combination for later use).



If not deactivated, the combined sounds associated with a user manipulation of the computer will be generated inside each experimental box by means of the associated loudspeakers and then enter in conflict with the running experiment producing animal stress and disruption of the experimental protocol.

2.9.3 Load Cell Calibration & Optimization

Before using the system for the first time and occasionally (when the apparatus is used by other experimenters, or when the apparatus is not used for long times), it is recommended to check the calibration of the load cell in each FREEZING and STARTLE experimental box.

The calibration process will ensure that the load cells associated with all the STARTLE boxes used in the study will work with the same sensitivity.

For carrying out the calibration process a calibrated weight of 20 g is needed.

2.9.3.1 Load cell optimization ("doing the zero")

The first step of the load cell calibration is "doing the zero". Doing the zero allows the weight transducer working with the maximum performance.

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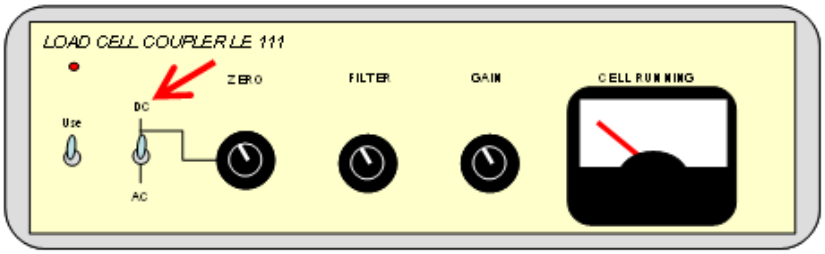
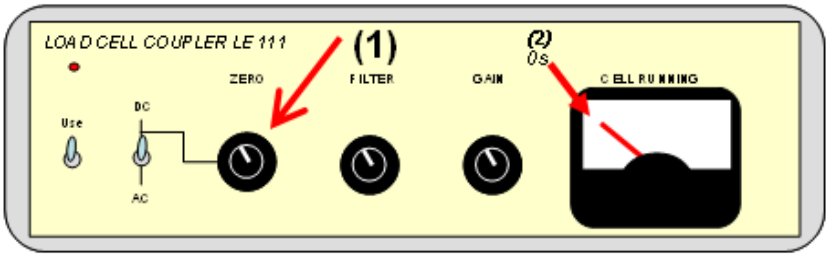
Once the load cell calibration process has been performed, we recommend the user checking the zero each day for each box before beginning the experiment. This is a very quick procedure.

The next operations have to be made for each experimental chamber.

- Select DC mode on the Load Cell Coupler LE-111 control unit (red arrow).

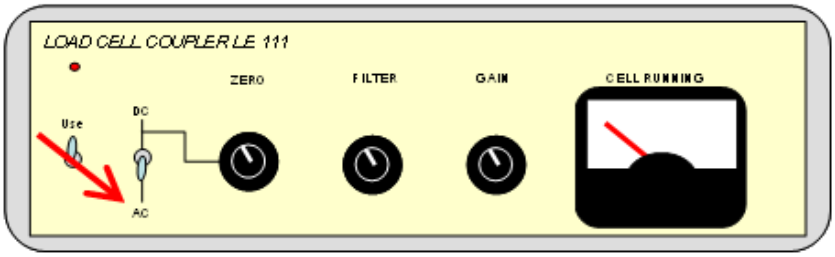


- Adjust the zero using the ***Zero*** button of the Load Cell Coupler LE-111 control unit **(1)** until the galvanometer indicates the zero value **(2)**.



A recommended way is to set the GAIN to a low value, turn the Zero knob (1) so that the needle moves towards the zero value (2). Increase the gain as you approach the Zero value. Finish the fine adjustment of the needle to the zero value with GAIN set to its maximum value.

Don't forget to set the Load Cell Coupler LE-111 control unit to the AC mode again for the acquisition of data during the experiment.



The zero can also be checked by visualizing the analogic signal received by the software. To do that:

- Select DC mode on the Load Cell Coupler LE-111 control unit as previously described.
- Start PACKWIN and continue the "Startle Test Elements.exp" (for Startle Reflex systems) or "Freezing Test Elements.exp" (for Fear Conditioning systems) experiment file located in the "STARTLE" or "FREEZING" folder of the default "Experiments" folder.

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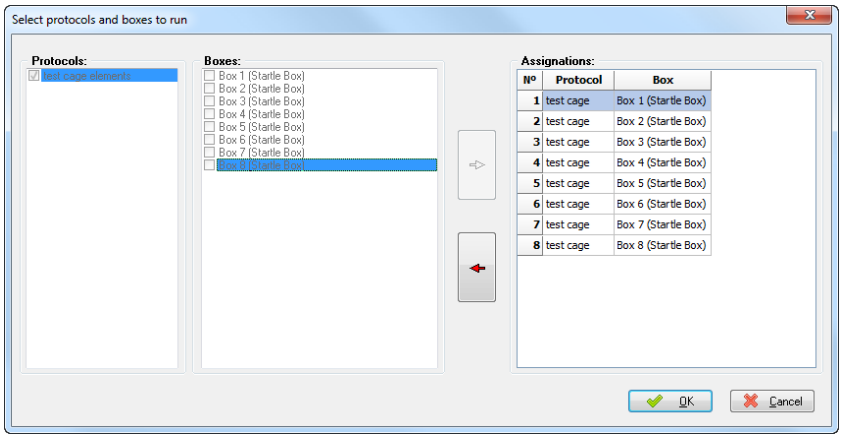
Please refer to the chapter 3.1.2 for more details on how to open an experiment file.

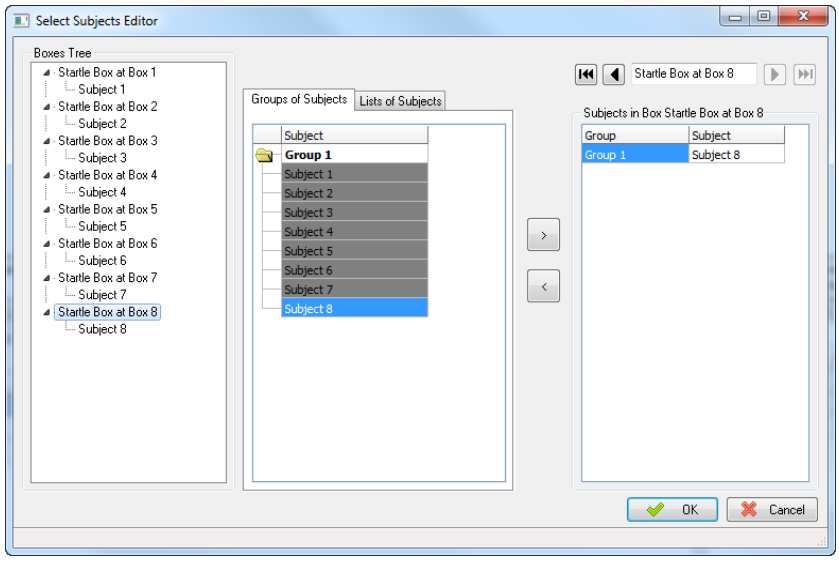
- Start a data acquisition session associating the protocol "Test cage elements" to each of the boxes.

  Please refer to the chapter 8.2. for more details on how to associate protocols and boxes.

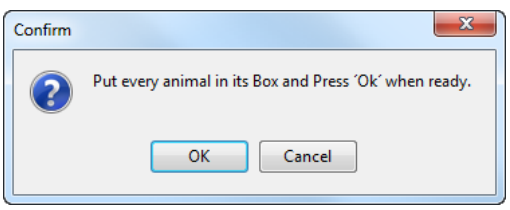


- Associate the experimental boxes with a subject.

  Please refer to the chapter 8.3.2.2. for more details on how to associate subjects to boxes.



- Start the data acquisition session but DO NOT put any animal into the boxes. Press OK.



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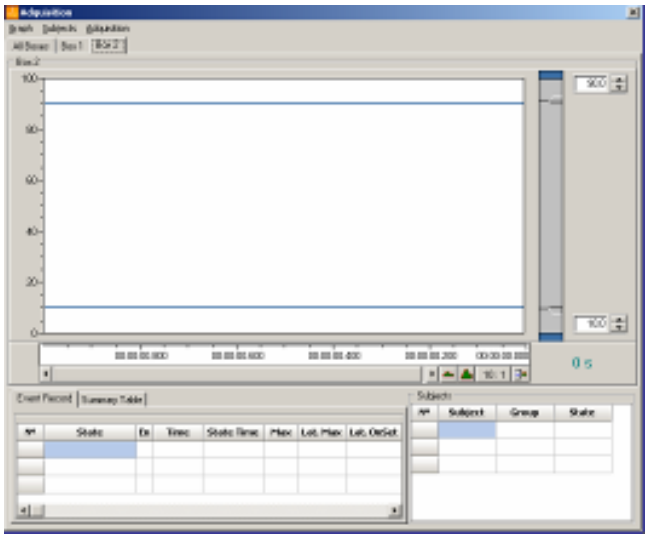
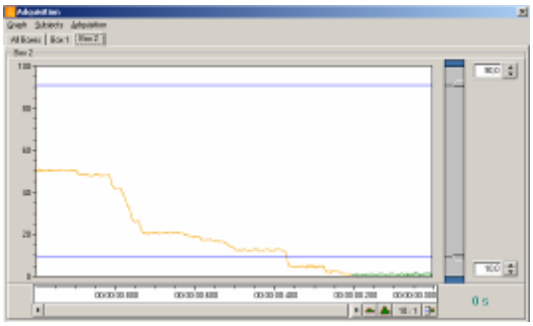
Please refer to the chapter 8.3.2.4. for more details on how to start the data acquisition process.

All the boxes will start synchronically and the acquisition of the activity signal from the weight transducers will begin.



When the zero is correctly set, the signal will appear aligned with the zero line (y axis = 0).

- Adjust the zero using the ***Zero*** button of the Load Cell Coupler LE-111 control unit as described previously and check the result on the screen. The signal has to reach the zero (0) value of the y axis.



- Repeat the process for all the boxes and its respective Load Cell Coupler LE-111 control unit.

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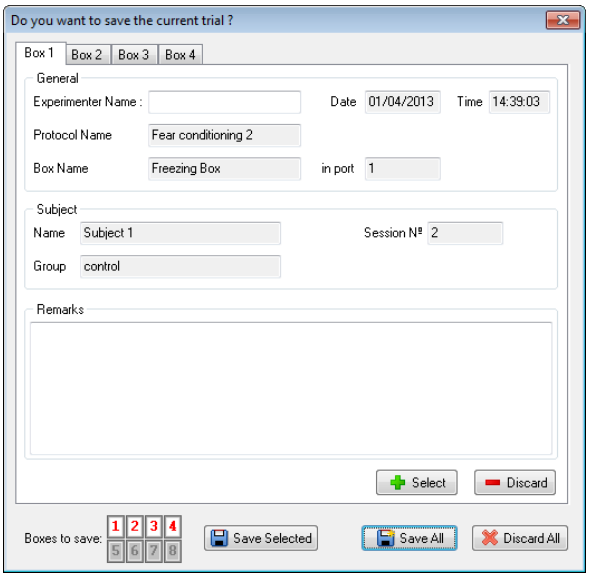
- Once the zero is adjusted, ***Stop*** the data acquisition session and press ***Discard All*** to avoid saving the session.

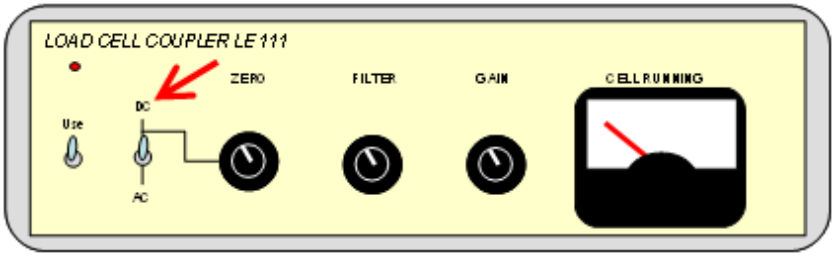


Don't forget to set the Load Cell Coupler LE-111 control unit to the AC mode again for the acquisition of data during the experiment.

2.9.3.2 Load cell calibration

The next operations have to be repeated for each experimental chamber.

- Select DC mode on the Load Cell Coupler LE-111 control unit (red arrow).



- Check the "zero" as described in the previous chapter (2.8.3.1.).
- Check the position of the grid floor: it has to fit the four position points of the weight transducer system.
- Put a 20 g weight on the grid floor.
- Set the GAIN button to the maximum value on the LE118.
- Read the value on the LE-111 galvanometer; it has to be 1.0 mA.
- If the displayed value is different from 1.0 mA, the system has to be recalibrated. The calibration process consists of adjusting the galvanometer value using the corresponding rear panel screw, ((1), "Load cell") until reaching the **1.0 mA** value (2).

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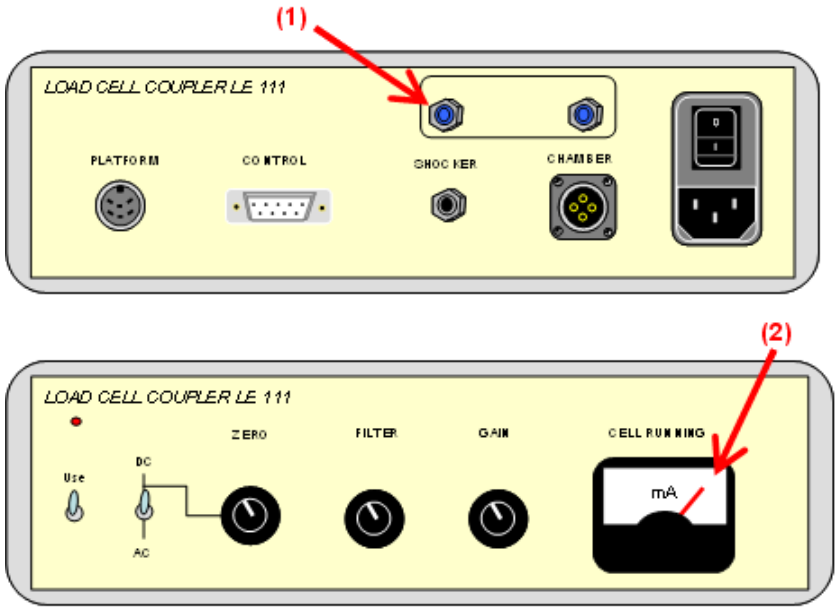
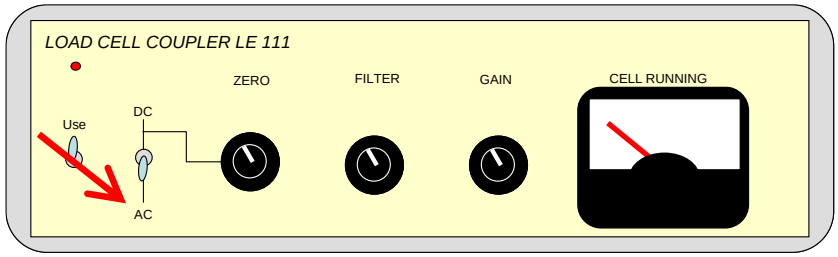


Don't forget to set the Load Cell Coupler LE-111 control unit to the AC mode again for the acquisition of data during the experiment.



The calibration process can also be visualized through the software. To do that:

- Select DC mode on the Load Cell Coupler LE-111 control unit as previously described.
- Start PACKWIN and continue the "Startle Test Elements.exp" (for Startle Reflex systems) or "Freezing Test Elements.exp" (for Fear Conditioning systems) experiment file located in the "STARTLE" or "FREEZING" folder of the default "Experiments" folder.

  Please refer to the chapter 3.1.2 for more details on how to open an experiment file.
- Start a data acquisition session associating the protocol "Test cage elements" to each of the boxes.

  Please refer to the chapter 8.2. for more details on how to associate protocols and boxes.

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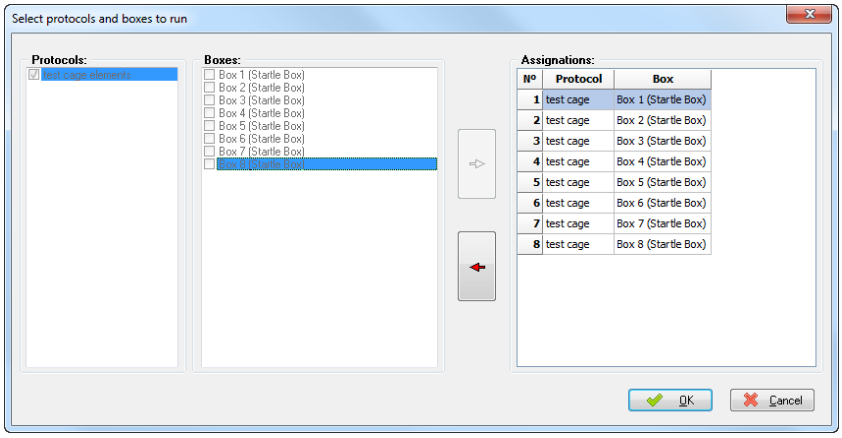
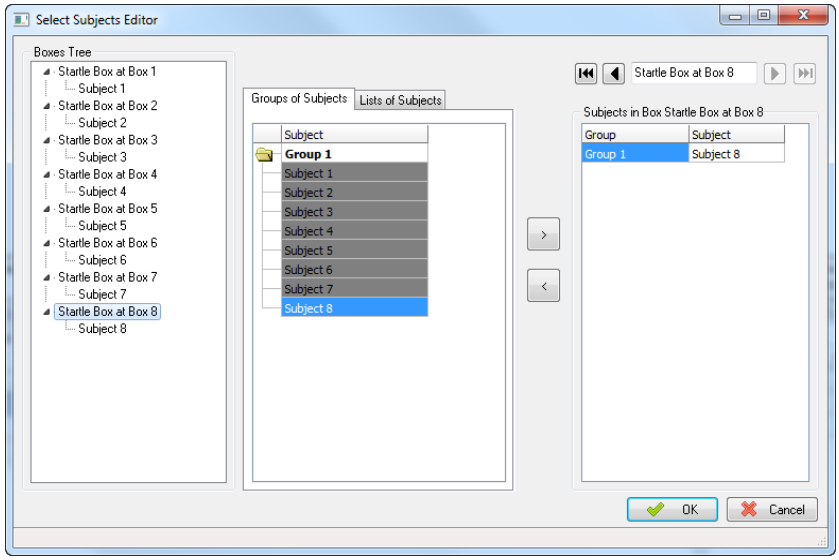
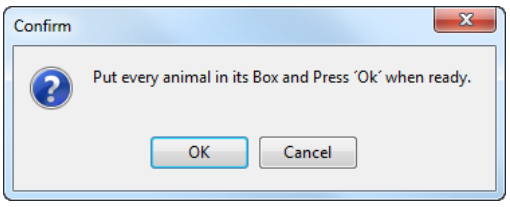


- Associate the experimental boxes with a subject.

  Please refer to the chapter 8.3.2.2. for more details on how to associate subjects to boxes.



- Start the data acquisition session but DO NOT put any animal into the boxes. Press OK.



  Please refer to the chapter 8.3.2.4. for more details on how to start the data acquisition process.

The data acquisition process will start at the same time in all the selected boxes and the activity signal from the weight transducers will be shown.

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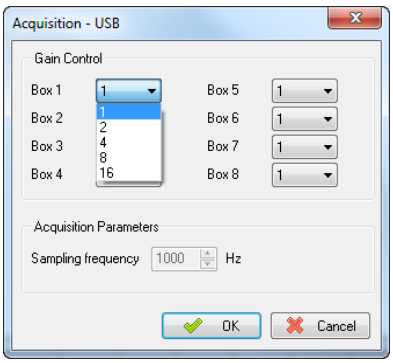
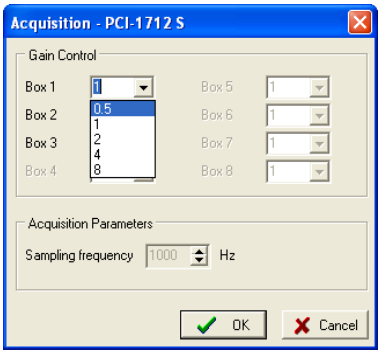
- Put a 20g weight on the experimental chamber grid and check the result on the screen. The signal has to reach to the same value in all the cages.
  - If your system is based in USB communication the signal has to reach to 100% when a gain of 16 is selected in the software. In that case, the gain values available are shown below:

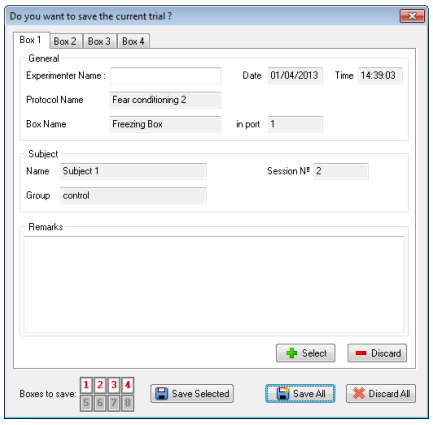


  - If your system is based in PCI communications (previous STARTLE/FREEZING hardware version), the signal has to reach to 100% when a gain of 8 is selected in the software. To select another gain value, select the **Acquisition – Configure** menu option. The available values are shown below:



- Adjust de value observed as described before using the corresponding screw on the rear panel of the LE-111 control unit.

  Once the value is adjusted, ***Stop*** the data acquisition session and press ***Discard All*** to avoid saving the session.



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Don't forget to set the Load Cell Coupler LE-111 control unit to the AC mode again for the acquisition of data during the experiment.

Systems based in PCI use a full-scale of ± 5 V whilst systems based in USB use a full-scale of ± 10 V, therefore the signal drawn by the software will be shown lower in USB systems.

In order to analyse in the same conditions, the software gain in USB-based systems must be double the PCI gain.

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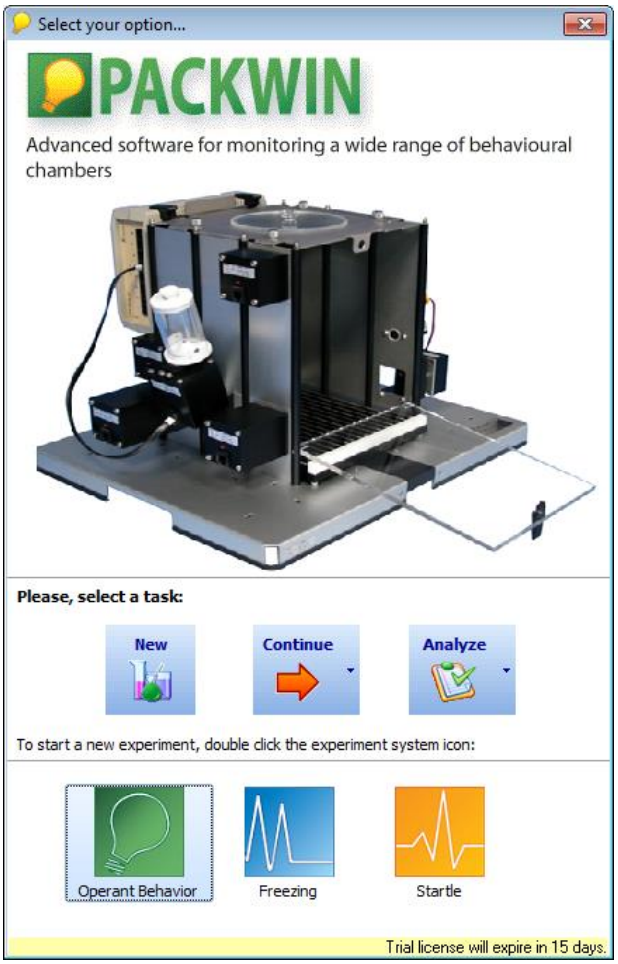
3 Starting With PACKWIN

In this chapter, we will describe the general features and menu provided by the software. Most of them are shared by the different PACKWIN modules. The specificities and content of each menu will be then detailed for each experiment module in different paragraphs.

3.1 Starting Assistant Panel

When PACKWIN is launched a **Starting Assistant** tool is show on the screen:



The **Starting Assistant** tool allows you to:

- Start a new experiment.
- Continue with a previously saved experiment.
- Analyze the data acquired in a previously saved experiment.

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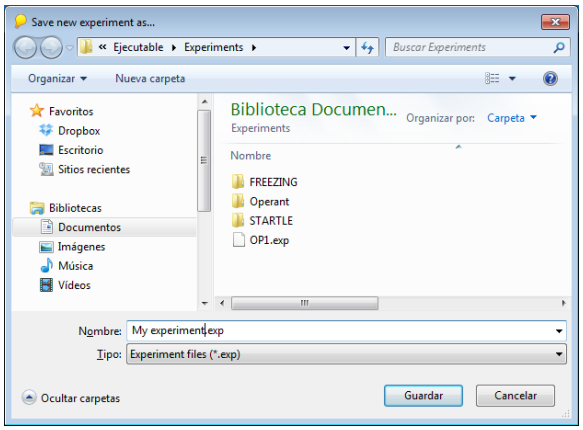
The Starting Assistant shows the list of available experiments depending on the PACKWIN module purchased:

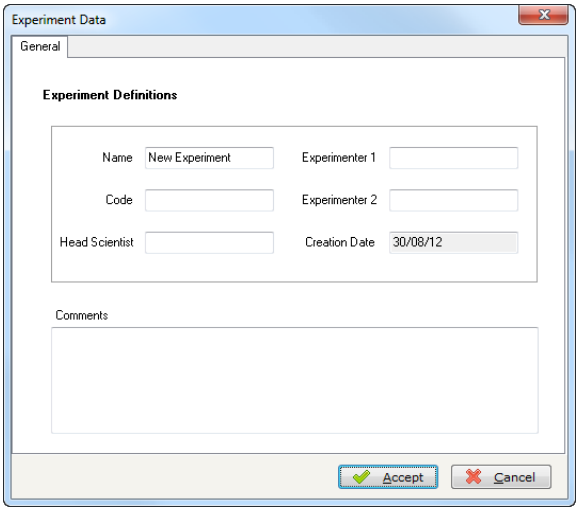
- OPERANT BEHAVIOR for PACKWIN-CS, HO, VT
- FREEZING for PACKWIN-CSFR
- STARTLE for PACKWIN-CSST

3.1.1 Creating a new experimental file

- Select the **New** task.
- Double click on the experiment type icon (OPERANT, STARTLE or FREEZING). Alternatively, you may also select the protocol first and then double click on the **New** task.
- Enter the name of the new experiment file and optionally select the folder in which the file will be stored. Then press the **Save** button.



- Enter details in the **Experiment Data** dialog.



This panel can be filled later by using the [Experimental data...] option of the [View] menu.

- Press the **Accept** button.

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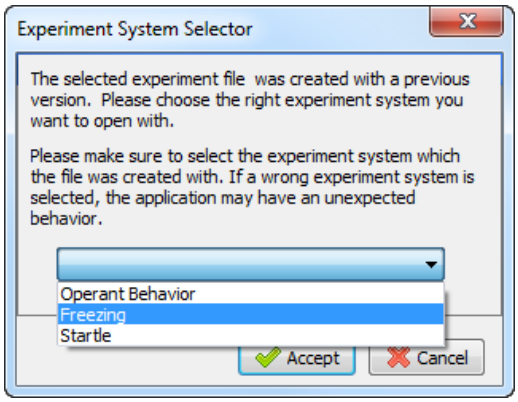
3.1.2 Continuing an experiment

To open an already existing experiment file and continue the experiment:

- Select **Continue**.
- Locate and select the desired folder and experiment file.
- Press **Open** to load the experiment file.
- If an experiment file generated with older versions of FREEZING and STARTLE software is selected, the next message will appear; then select the corresponding experiment type (Freezing or Startle) to carry out from the list and press the **Accept** button.



It is critical to select the experiment type related to your previous experimental file. Otherwise, the application may have unexpected behavior within the rest of the functionalities. In case of doubt, do this operation using a copy of your original experimental file.

3.1.3 Analyzing an experiment

To analyse the sessions contained in an already existing experiment file:

- Select the **Analyze** task.
- Locate and select the desired folder and experiment file.
- Press the **Open** button to load the experiment file.
- If an experiment file generated with older versions of FREEZING and STARTLE software is selected, the next message will appear; then select the corresponding experiment type (Freezing or Startle) to carry out from the list and press the **Accept** button.

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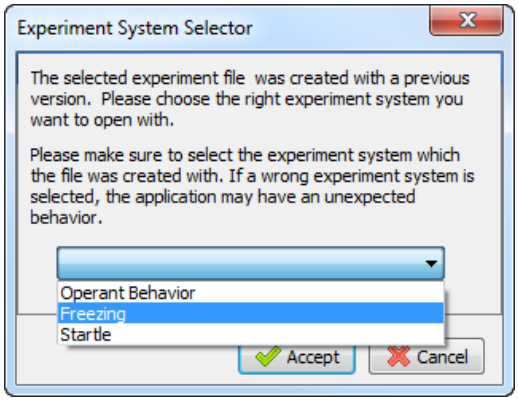

It is critical to select the experiment type related to your previous experimental file. Otherwise, the application may have unexpected behavior within the rest of the functionalities. In case of doubt, do this operation using a copy of your original experimental file.

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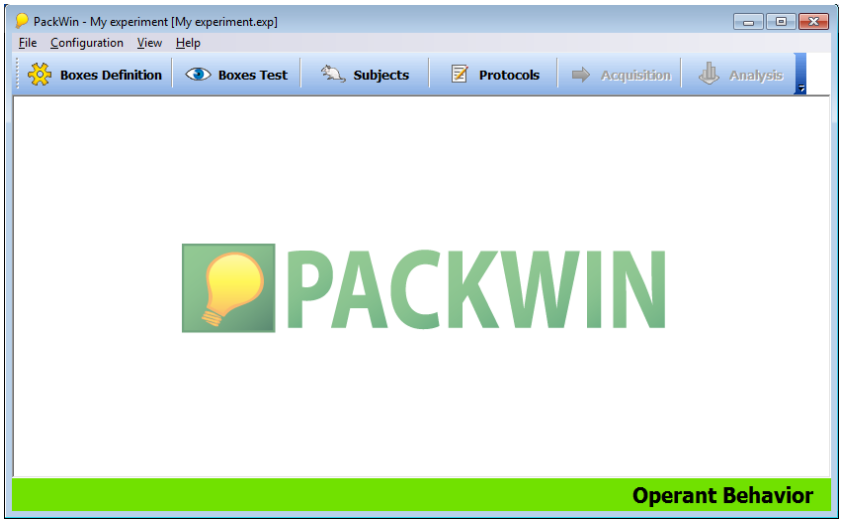
3.2 PACKWIN main window



Once the new experiment is initiated, the main window of PACKWIN is shown with the following elements:

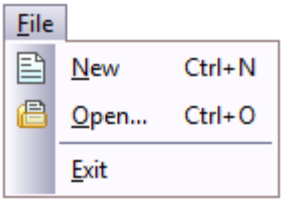
3.2.1 Title bar

The title bar shows the name of the application, the name of the current experiment and the name of the file created.

3.2.2 Main menu bar

The main menu bar contains all the functions that are available within the application arranged into 4 sections: File, Configuration, View, and Help.

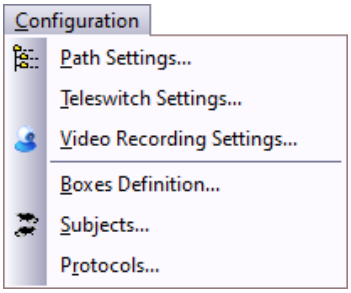
3.2.2.1 File menu



The **File** menu provides options for managing the experiment files:

- **New**: create a new experiment file.
- **Open**: open an already existing experiment file.
- **Exit**: close the experiment file.

3.2.2.2 Configuration menu



The **Configuration** menu provides access to the following configurations:

- **Path Settings…:**

  By default, PACKWIN automatically stores the Experimental files, exported protocol files, and Report files in a specific folder located in the PACKWIN program files folder.

  It is a good practice to change the default paths by those which are more suitable for the experimenter's needs.

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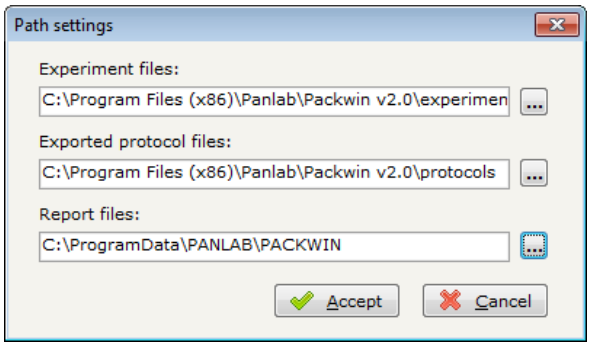
To do that:

- Select Configuration – Path settings menu option.
- Press the ... button associated to the requested field.
- Locate and select the desired folder.
- Press **Accept** button.



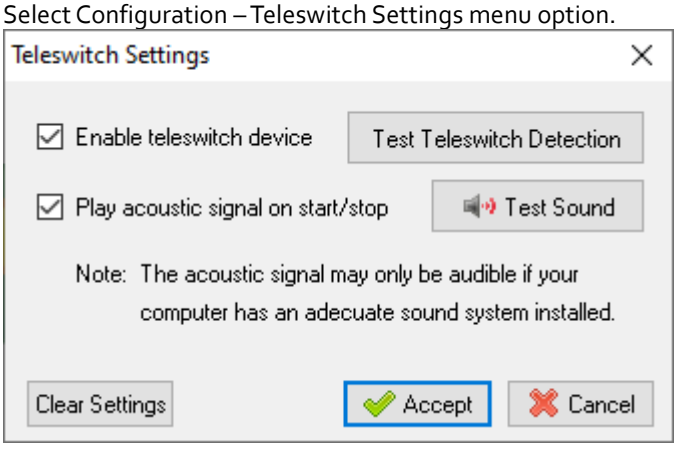
This information is stored within the experiment file so this task should be done per each new experiment created.

- **Teleswitch Settings…**

By default, the use of a remote control to Start/Stop the Data Acquisition is disabled. Select this option to enable it and to execute the detection test.

To do that:

- Select Configuration – Teleswitch Settings menu option.



- Check the box **Enable teleswitch device** if you want to enable the teleswitch device.
- Click button **Test Teleswitch Detection** to open the assistant that allows testing the teleswitch detection and registering the device. The assistant consists of two steps:
  - The first step requires to press the key for letter "p" at the standard keyboard.

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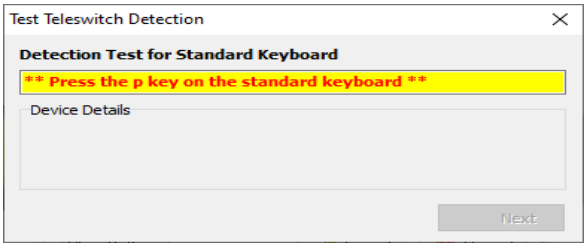

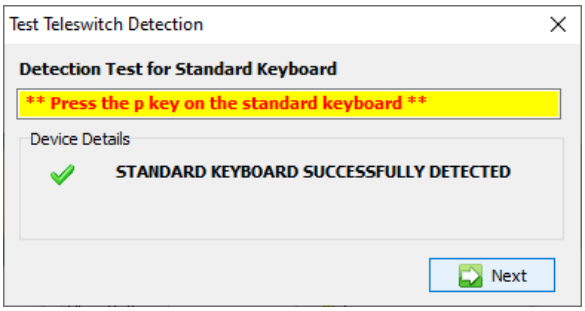

- The second step requests to press the key defined for Start or Stop Data Acquisition at the teleswitch (Please refer to the Chapter 2.8 for detailed information of the keys used in different Teleswitch versions).

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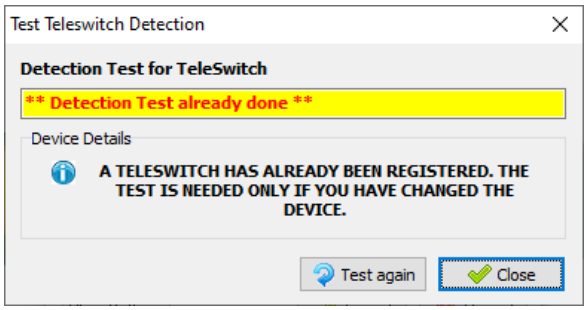
Besides, this step verifies that the identifier of the standard keyboard is different from the identifier of the teleswitch, and if not, an error message is shown, and the device is not accepted.

Once a teleswitch was detected and registered, it is possible to change the device for a different one. To do that, click on button **Test again** and repeat the previous steps.



Execution of this detection test is required if the teleswitch has been.

- Check the box **Play acoustic signal on start/stop** to make the computer play an acoustic signal (beep) whenever the trial starts or stops using the teleswitch unit.

The **Test Sound** button will allow you to test the sound playing before starting any trial.

This useful tool will help you to make sure that the teleswitch unit is working fine but may affect the answer of the subjects. Please check this box only if it does not affect within your specific experimental conditions.

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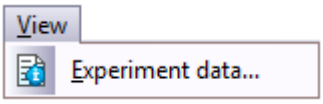
The acoustic signal is played by means of the sound system of your computer so please make sure the sound card and the speakers are properly installed and turned on.

- The selected Teleswitch settings will be available for new experiments. To reset the Teleswitch settings, click the button **Clear Settings**.

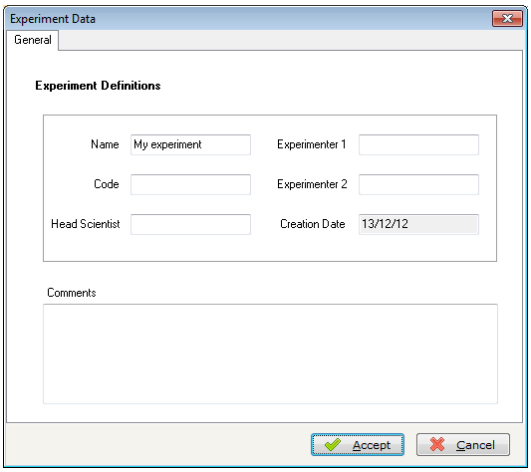
- **Video Recording Settings…:** set up the automatic start-stop of video recording through the external application Record-It! Media. See Chapter 12 for more details.
- **Boxes definition…**: configure the boxes used in the experiment. The box definition panel will depend on the experimental module used (see the chapter of each specific module for more details).
- **Subjects…**: configure the subjects used in the experiment. See Chapter 6 for more details.
- **Protocols…**: configure the protocols used in the experiment. See Chapter 7 for more details.

3.2.2.3 View menu

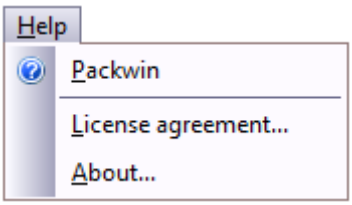


The **View** menu provides the following option:

- **Experimental Data…**: use this panel for entering general information about the experiment contained in the experimental file. The user can modify this information at any moment. All the sections are available for free text editing, except the **Creation Date** section showing the creation date of the file (which is fulfilled automatically by the system).



3.2.2.4 Help menu



The **Help** menu provides the following options:

- **PACKWIN**: open the pdf of the PACKWIN User's Manual provided with the system.

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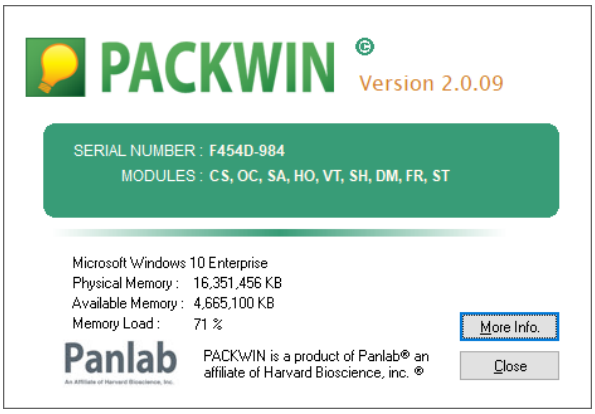
- **About...**: provides information about the purchased system that should be provided to Panlab team in case technical support is required:
  Software serial number.
  Purchased PACKWIN modules.
  Computer operative system and available memory.



In this panel, the **More Info.** button provides additional useful information about Panlab.

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3.2.3 Experimentation Assistant bar

The **Experimentation Assistant bar** is designed to give the experimenter a quick way to access to the operations associated to the main steps of a typical experimental process.

This bar is designed in a way that only the currently allowed operations are active. The main tasks and the suggested order to be executed are the following:

1		To detect and configure the boxes included in the system.
2		To verify operation of the module inputs and outputs in each box
3		To manage the experimental subjects and groups database.
4		To manage the experimental protocols to be used.
5		To execute and register experimental sessions.
6		To generate analysis reports of registered sessions.

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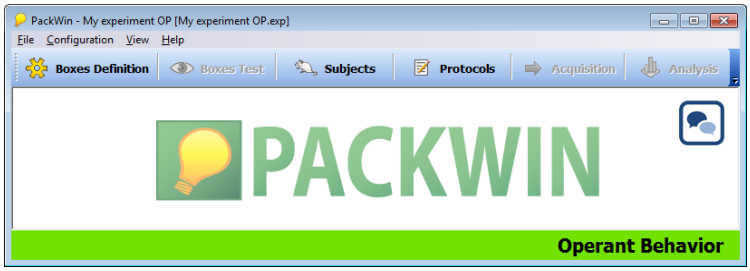
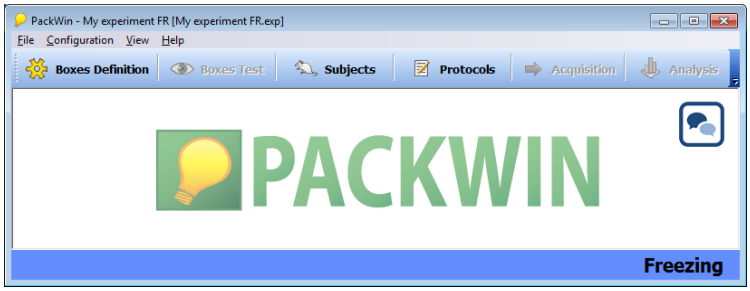
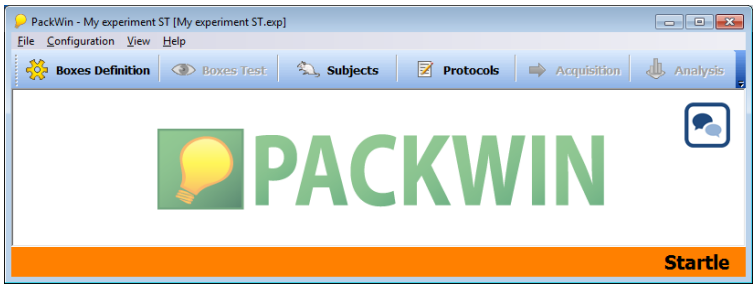
3.2.4 Experiment identification bar

A bar indicating the PACKWIN module choosen by the user is located in the bottom right side of the main window:

- Operant Behavior for PACKWIN operant modules
- Freezing for PACKWIN FREEZING module
- Startle for PACKWIN STARTLE modules







4 Boxes definition

This section allows detecting and defining the boxes connected to the system.

Press **Boxes definition** button and select the mode of PACKWIN to be used between the two options proposed:

- **USE REAL CONNECTED BOXES** when using the real boxes connected to the system.
- **USE VIRTUAL BOXES** when using simulated boxes. The virtual boxes configuration can be used for running the PACKWIN software on a computer to which real boxes are not connected (e.g. for creating and checking protocols or for teaching purposes).

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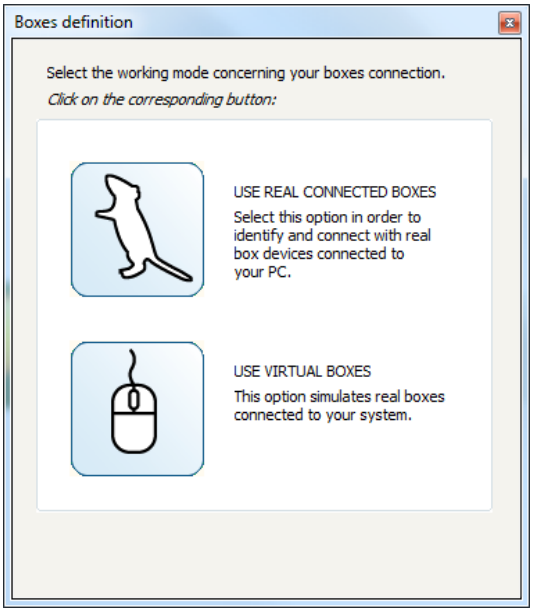


4.1 Real boxes definition

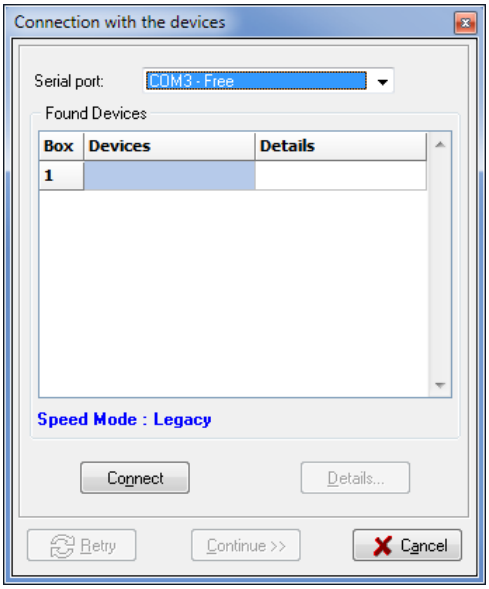
The Real boxes option is not available in the PACKWIN Trial version. A valid PACKWIN USB license key must be connected to be able to work with real boxes.

The configuration of the Real box definition differs among the different PACKWIN modules used.

4.1.1 PACKWIN Operant Module (CS)

4.1.1.1 Checking the connections to the boxes

When the Real boxes option is selected, the following dialog is shown.



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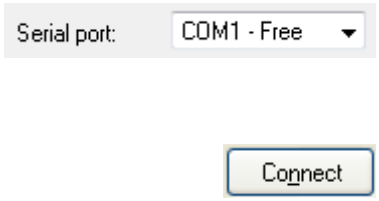
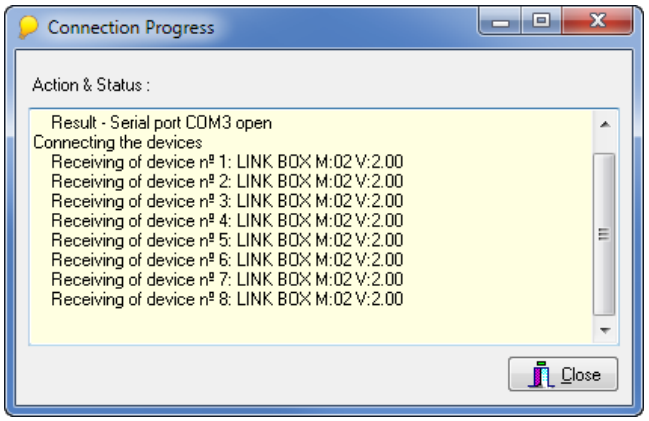
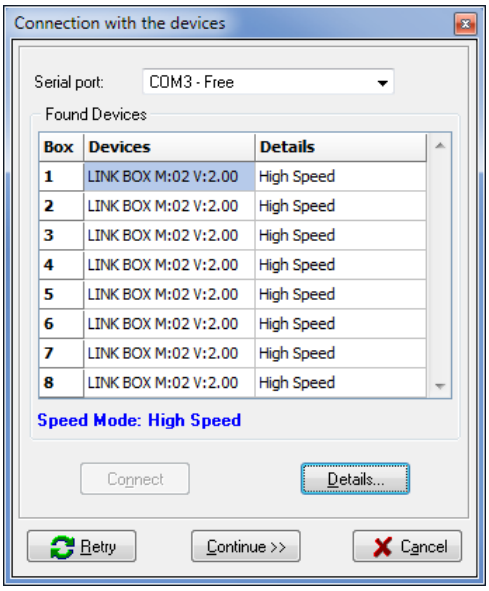


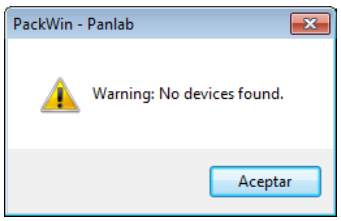
- To connect to the boxes:
  - Select the communication port in the **Serial port** section (it indicates the computer port number where the first Link box is connected).
  - Press the **Connect** button: for detecting all the Link boxes connected to the selected serial port.



Once detected the following panel is shown with the list of all the detected devices.



- If the system failed to detect the connected devices the following message appears:



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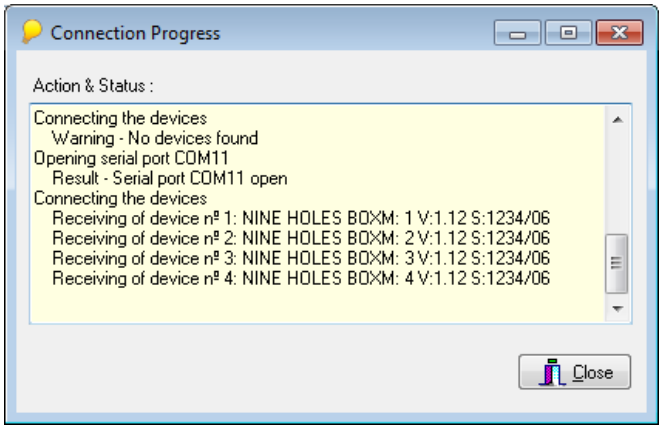
In that case, please check the following points:

- Link boxes connections are established according to the corresponding hardware User's Manual.
- The first Link box is connected to the computer using the correct cable and RS232/USB adapter sent with the system.
- The drivers of the RS232/USB adapter have been correctly installed.
- The selected serial port is correctly chosen.
- The serial port is not used/blocked by another running application.

- After checking these points, press the **Retry** button to refresh the detection process. If the error message still appears, contact your dealer for technical support.

- Press the **Details** button for showing the Connection Progress panel.



Press **Continue** button to access the **Box Configuration** panel.

SPEED MODE: HI-SPEED OR LEGACY

PACKWIN can handle both the current Linkbox01 and the new Linkbox01-HS, which works using a high speed communication protocol. When the Linkbox is detected, the speed mode shown in the "Connection with the devices" panel is marked as "High-Speed".

Depending on the configurations, the Panlab LINKBOX01 HS would work at the High-Speed or Legacy Mode.

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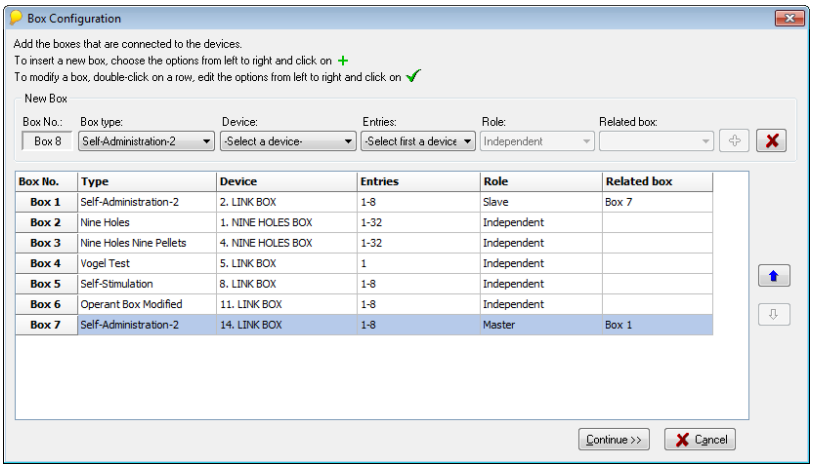
The High Speed mode is only guaranteed when the daisy chain of Linkbox is only composed of High-Speed Linkbox (Linkbox01HS) and combined with the High speed RS232USB converter (CONRS232USB-HS). See table below.

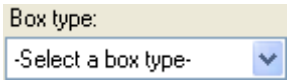
Linkbox model	Rs23USB Converter	Speed Mode *(shown on PACKWIN panel)*	Speed
1 or daisy chain of LINKBOX01 HS	CONRS232USB-HS	High-Speed	High-Speed
1 or daisy chain of LINKBOX01 HS	CONRS232USB	High-Speed	Legacy<speed<High Speed (not recommended)
1 or daisy chain of LINKBOX01	CONRS232USB	Legacy	Legacy
Mixed daisy chain of LINKBOX01 HS and LINKBOX01	CONRS232USB-HS	Legacy	Legacy

4.1.1.2 Boxes configuration

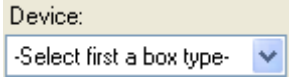
The following dialog (but empty!) to configure the operant conditioning boxes will be shown after connecting to the devices:



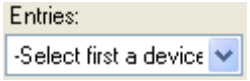
- To **add** and configure a new box:
  - Select a box type from the **Box Type** drop down list. After this, the application will search for devices that are compatible with the box type you selected and are not yet occupied. The box types available are the ones contained in the specific customer box configuration file.



  - Select the device from the **Device** drop down list which is connected to the box. After the device is selected, the application will list the group of entries available for the selected device.



  - Select the group of entries from the **Entries** drop down list in which the box is connected. The number of entries in a group of entries depend on the selected box type because different types



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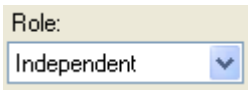
of boxes require different number of entries. A box may occupy only one entry or even the whole box.

PACKWIN v2.0 is able to execute a protocol in yoked mode without making any physical change in wiring cages, just by setting the yoked role for each cage.

- To set yoked boxes, follow the next instructions:
  - Select the role of the box. A box can play the following roles when executing a protocol:

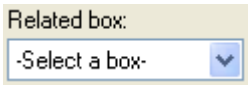
    

    Independent: This is the default value. In this mode, the box will execute a protocol without affecting and being affected by another box.

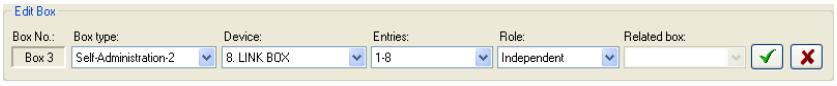
    Master: The box will execute the assigned protocol without been affected by another box. All stimuli received by the master box, will also be presented inalterably in the associated slave box.

    Slave: The box is cage-related to a master. The stimuli presented in this box are originated from the master's protocol execution. The behaviour of the subject in a slave box does not affect the evolution of the protocol at all.

  - Select the yoked slave box related to this master box. This option will be available only if a Master box exists in the table and will list all the available independent boxes. After choosing a box number and clicking the Add button, the selected related box role will be marked as Slave of the corresponding master box.

  - After selecting all the five elements, the **Add** button will be enabled. Click on to add the box to the list. The number of the box is consecutive from 1 to N. To change the number of the box, use the arrows and and then move the box up or down the list respectively.
- To **edit** an existing box:
  - Double click on the box you wish to edit. The configuration of the box will appear on the drop down lists in the header of the dialog.

  - Edit the settings the same way as if a new box is added and click on to apply the changes or on to cancel the edit.
  - To modify the yoked role of a slave box, first edit its master's related box and set its role to independent. Then edit and select the role for the desired box.
- To **delete** a box, select the box you wish to delete and click on the **Delete** button .

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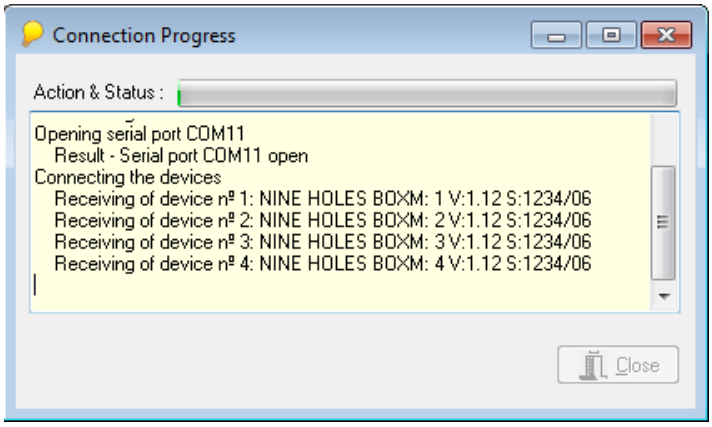
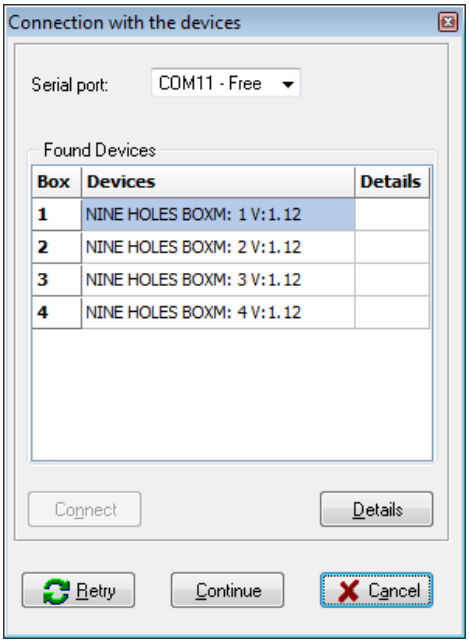
After configuring your operant conditioning boxes, press the **Continue** button. The settings will be saved so it not necessary to re-configure the boxes the next time you enter into the **Box Configuration** panel.

4.1.2 PACKWIN Operant Nine Hole Module (HO)

4.1.2.1 Checking the connections to the boxes

Same as the PACKWIN CS module, see details in chapter 4.2.1.1. The following panel is shown during the connection process.





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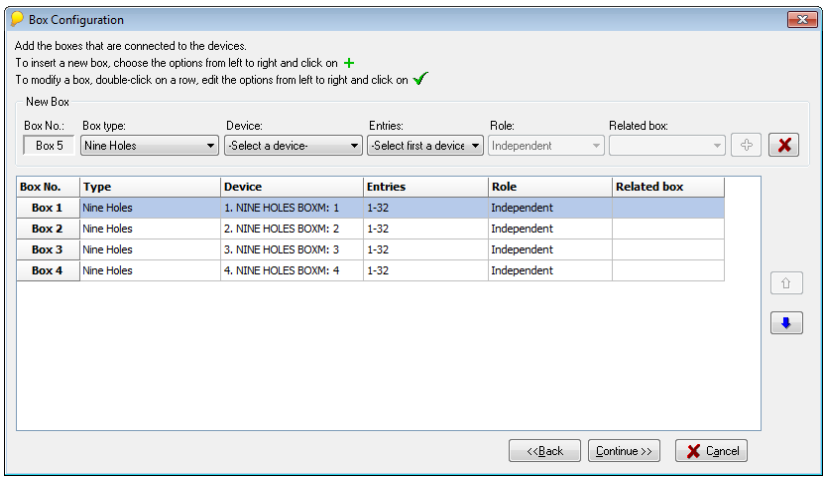
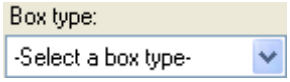
4.1.2.2 Boxes configuration

Same as the PACKWIN CS module, see details in chapter 4.2.1.2.

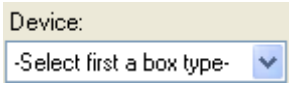
The following dialog (but empty!) to configure the 5/9 hole boxes will be shown after connecting to the devices:



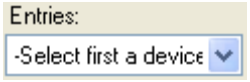
- To **add** and configure a new box:
    - Select a box type from the **Box Type** drop down list. The **Nine Holes** option is available for 5/9 hole boxes.



    - Select the device from the **Device** drop down list which is connected to the box. After the device is selected, the application will list the group of compatible interfaces Linkbox detected. Here, the **NINE HOLES BOX: n** will be shown (n = Linkbox ID).



    - Select the group of entries from the **Entries** drop down list in which the box is connected. For 5/9 hole the only available option is **1-32**.



    - In the PACKWIN-HO module, the yoked capabilities are not available, so the Role column editing is disabled.

See chapter 4.2.1.2. for detailed information about all the functions provided by the Box configuration panel (edit an existing box configuration, delete a box configuration etc..).

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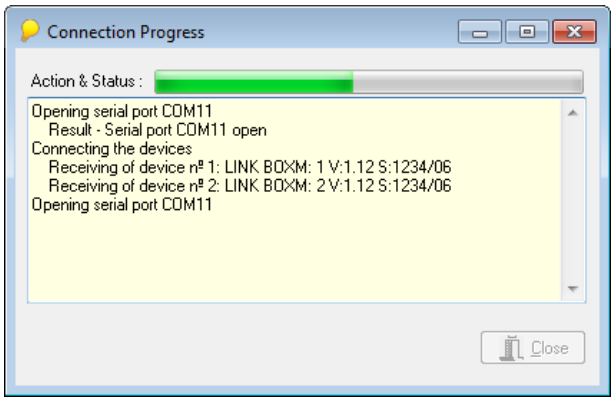
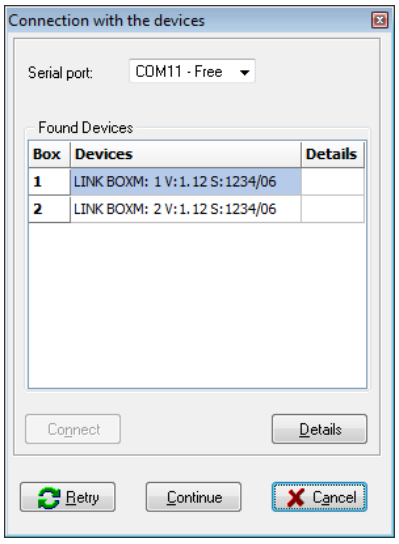
4.1.3 PACKWIN Operant Vogel Test Module (VT)

4.1.3.1 Checking the connections to the boxes

Same as the PACKWIN CS module, see details in chapter 4.2.1.1. The following panel is shown during the connection process.



The following panel is shown during the connection process.



As a difference with standard operant boxes, one Linkbox can connect up to 8 Vogel test boxes.

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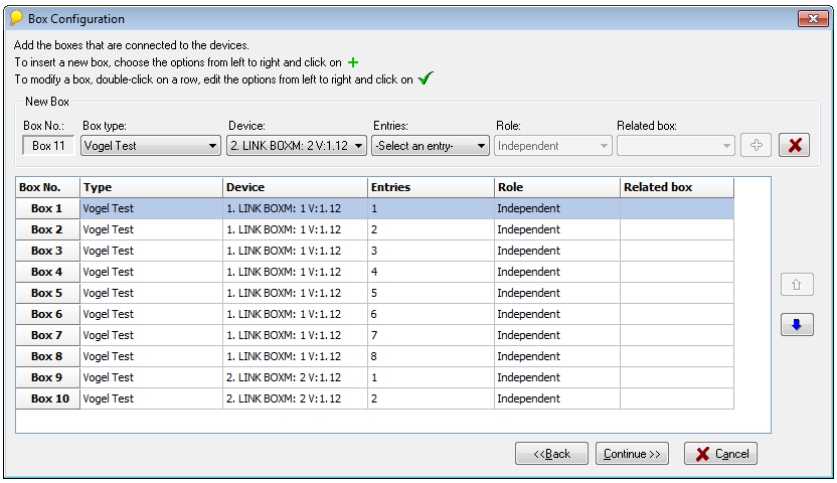
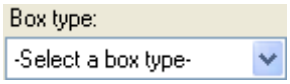
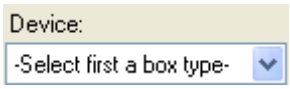
4.1.3.2 Boxes configuration

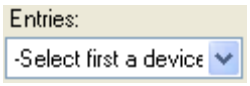
Same as the PACKWIN CS module, see details in chapter 4.2.1.2. The following dialog (but empty!) to configure the Vogel test boxes will be shown after connecting to the devices:



- To **add** and configure a new box:
    - Select a box type from the **Box Type** drop down list. The **Vogel test** option is available for Vogel test boxes.
    - Select the device from the **Device** drop down list which is connected to the box. After the device is selected, the application will list the group of compatible interfaces Linkbox detected. Here, the **LINK BOX: n V: 1.12** will be shown (n = Linkbox ID; V:1.12 is the version of the internal firmware).
    - Select the group of entries from the **Entries** drop down list in which the box is connected. For Vogel test, the link box can connect up to 8 Vogel test boxes, so the entries available for each box are labelled from number 1 to number 8 and has to be selected by the user for each box.
    - In the PACKWIN-VT module the yoked capabilities are not available, so the Role column editing is disabled.







See chapter 4.2.1.2. for detailed information about all the functions provided by the Box configuration panel (edit an existing box configuration, delete a box configuration etc..).

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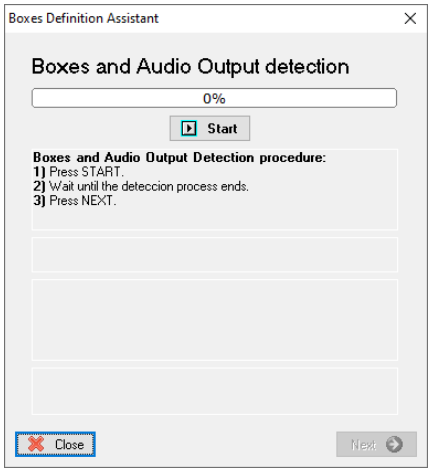
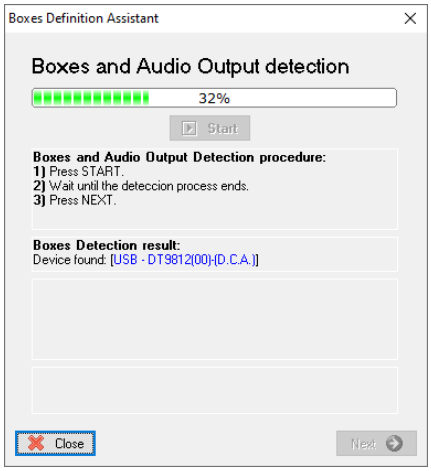
4.1.4 PACKWIN Freezing and Startle Module (CSFR and CSST)

4.1.4.1 Boxes & audio device detection

In the case of the fear conditioning system, a specific **Boxes Definition Assistant** is provided to identify and configure the boxes connected to the computer and the installed sound system.



Press the **Start** button to identify the boxes connected and the sound system available in the computer.



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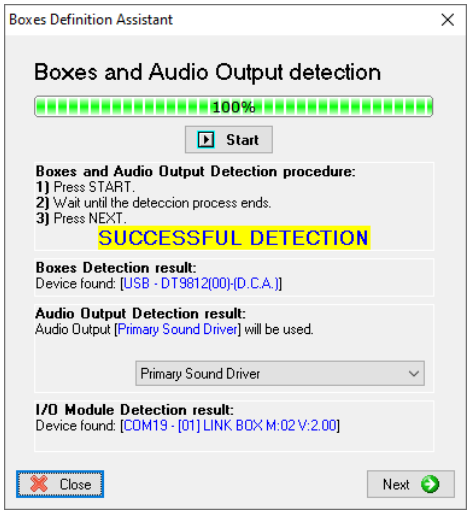
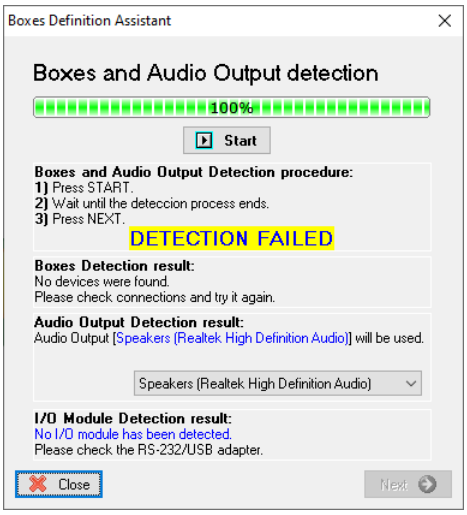
Once the process finishes successfully, a panel is shown with the following data:

- SUCCESSFUL DETECTION message.
- The "USB" LE118 device has been detected in the **Boxes Detection result** section.
- An **Audio output** (which name will depends on your system) has been detected in the **Audio Output Detection result** section.



- An **I/O Module (Linkbox01)** (of which communications parameters will depend on your system) has been detected in the **I/O Module Detection result** section.

If the detection fails, the assistant asks for checking the connections and trying the process again. In that case, the next steps are not available.



This assistant provides additional steps to:

- Check the sound card voltage
- Calibrate and optimize the sound card
- Calibrate the loudspeakers

Press the **Next** button to continue the sound calibration process.

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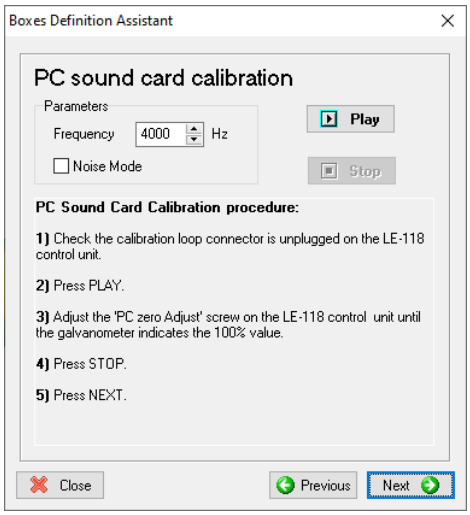
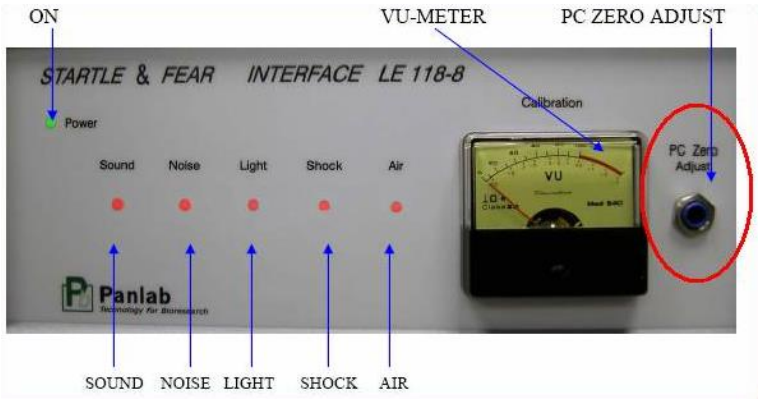
4.1.4.2 Check the sound card potency

The voltage sound card provided by the computer that will be used for the experiment must be checked as a first step of the sound calibration. Follow the next instructions:

- Please check that the calibration loop connector is unplugged on the LE-118 control unit before testing the voltage. If the loop connector is plugged in, the LE-118 control unit will not receive the sound signal from the computer.
- Press the **Play** button in the "PC sound card calibration" step of the **Boxes Definition Assistant.**



- Adjust the **PC Zero Adjust** potentiometer so that the Vu-meter provided on the front panel of the LE118 unit indicates 100%. The needle will be between the red and black section.



T

If the value the Vu-meter doesn't reach 100%, adjust the "PC Zero Adjust" screw in the front panel of the LE118 unit by turning it right or left until the value increases to the requested 100% value.

Press the Stop button for switching off the sound.

Press the **Finish** button to finalize with the boxes definition assistant for Fear Conditioning systems.

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If the 100% value still cannot be reached after this operation, it is possible that the sound card provided with the computer does not fulfil the requirements for its use for the system (see chapter 2.1. for system requirements). Indeed, if the value does not reach to 0.5V, then the sound card does not have enough power for working with the STARTFEAR System and must be replaced by other model. This may happen with the sound card provided with many laptops, so a desktop is recommended for a reliable use of the PACKWIN CSFR module.

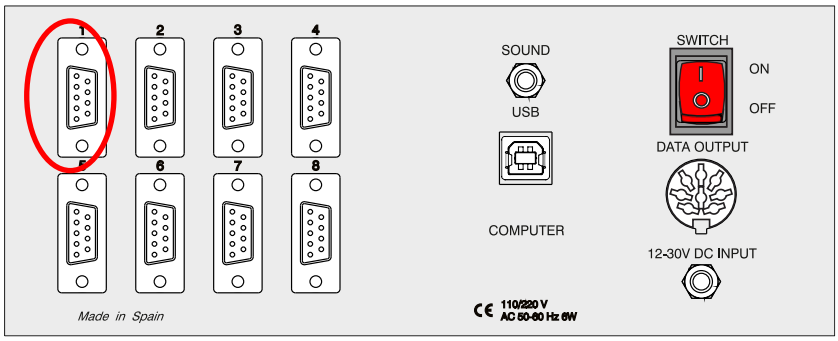
If you do not have any LE118(-8) to test the voltage of the computer sound card, the measurement can be performed with a voltmeter (see next chapter for details).

4.1.4.3 Audio performance calibration (only for startle CSST module)

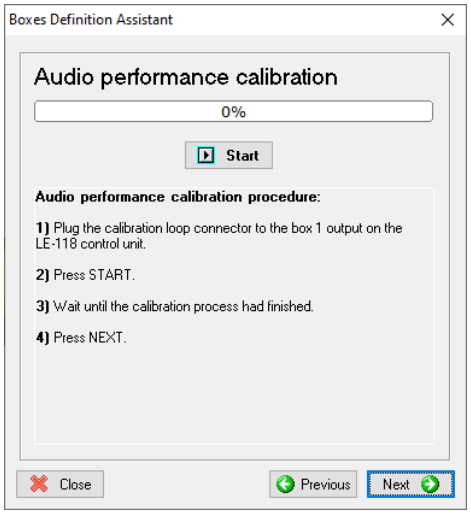
As STARTLE experiments need generation of very short sound stimuli, a specific step is required to calibrate and optimize the timing of the sound card functioning.

To do that:

- Plug the calibration loop connector to the port 1 in the LE-118 control unit.



- Press the **Start** button in the "Audio performance calibration" step of the **Boxes Definition Assistant.**



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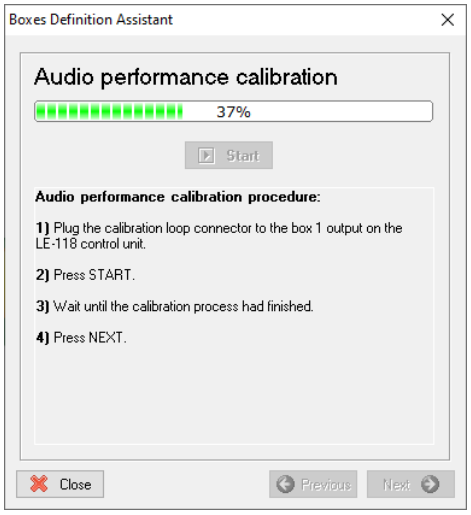
Wait for the audio calibration process to finish. During the audio calibration process, the calibration loop connector lets the computer "hear" the same sound signal it generates so that any possible delay in the timing of the sound produced by the sound card can be precisely estimated and compensated.



Press the **Next** button to continue with the assistant to calibrate the loudspeakers installed in the boxes.

4.1.4.4 Loudspeaker calibration

The loudspeaker calibration ensures the homogenization of the intensity of the sound between the experimental chambers when the system is used with more than 1 box.

The loudspeaker calibration is a procedure that has to be performed for each experimental chamber.

- Install the microphone of the decibel meter in the first experimental chamber.

We recommend installing the microphone of the decibel meter in the same place and same position in all the chambers in order to compare the values obtained between the chambers.

- In the **"Box(es) sound calibration"** step of the **Boxes Definition Assistant,** press the **Play** button to generate the calibration sound of 4kHz and 120 dB.

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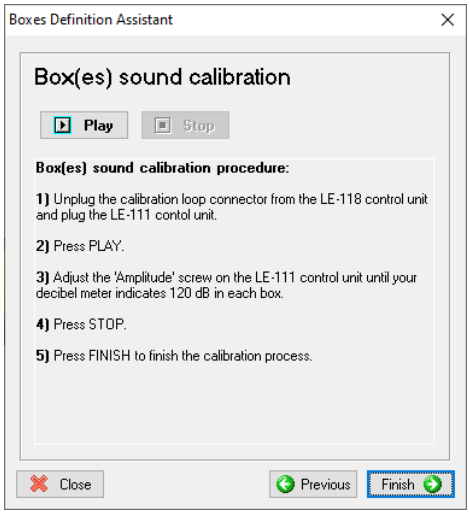
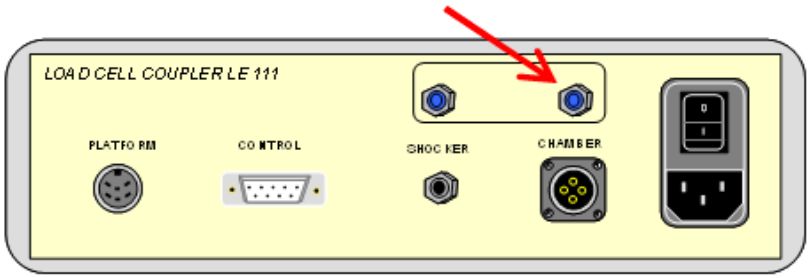


- Adjust the "Amplitude" screw on the rear panel of the LE-111 Control Unit (red arrow) until getting the 120 dB value in the decibel meter.



- Press the **Stop** button for switching off the calibration sound.
- Repeat the operation until getting the same value (± 3 dB) in all the chambers.

Press the **Finish** button to finalize the boxes definition process for Startle Reflex systems. Please continue with the load cell optimization process described in the next chapter.

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4.2 Virtual Boxes Definition

In the Virtual boxes mode, the PACKWIN software can be used without any connection to a real box. This is a useful tool (1) for checking the protocols without having to interrupt an ongoing experiment and (2) for teaching purposes.

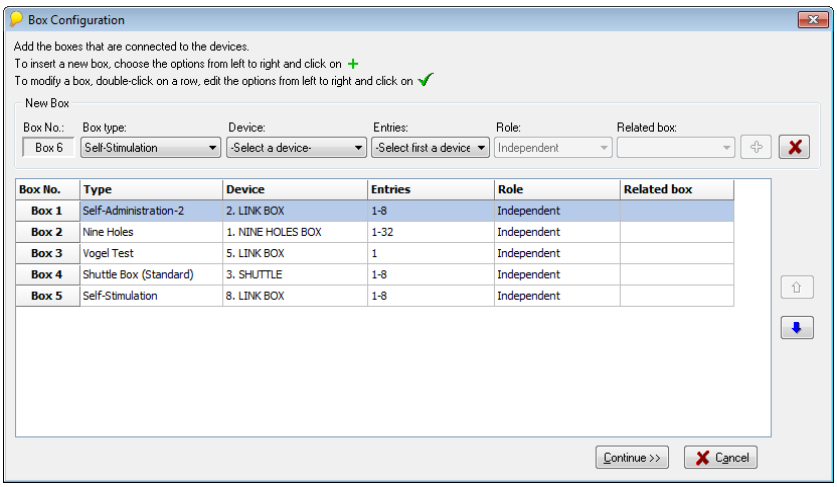
The box types available are the ones contained in the specific customer box configuration file (except during the trial period in which a demo configuration is provided).

The configuration of the Virtual boxes definition differs among the different PACKWIN modules used.

4.2.1 PACKWIN Operant Modules (CS)

In the Virtual boxes mode of the PACKWIN operant modules, the **Box Configuration** panel is directly shown for virtual boxes configuration.

The box types available are the ones contained in the specific customer box configuration file (except during the trial period in which a demo configuration is provided with different examples of experimental boxes).



Box configuration with virtual boxes is the same than with real boxes (please refer to the previous chapter 4.2.1.2. for more details).

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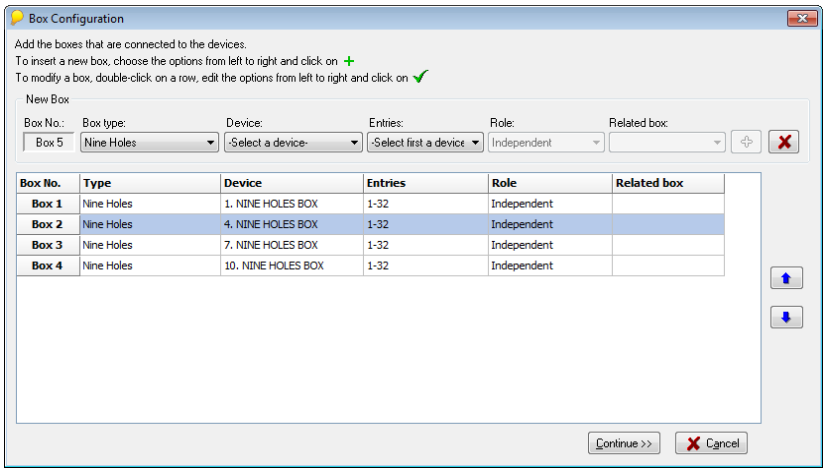
4.2.2 PACKWIN Operant Nine Hole Module (HO)

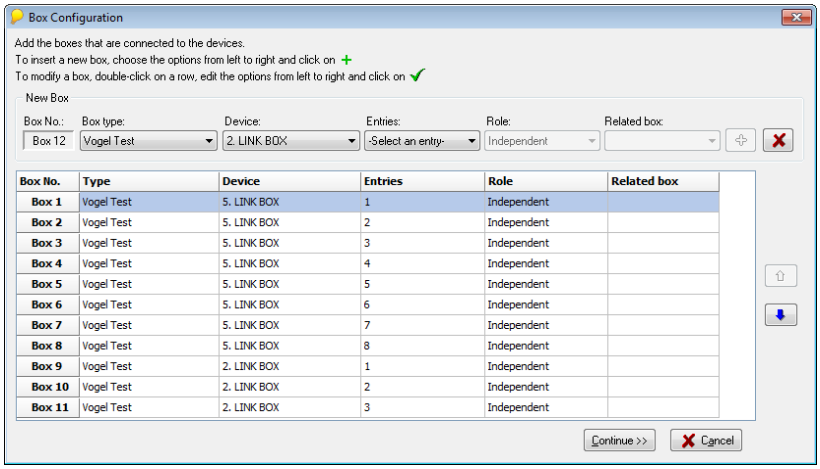
In the PACKWINHO, the only available box configuration available is the Nine Holes box configuration.



Box configuration with virtual boxes is the same as with real boxes (please refer to the previous chapter 4.1.2.2. for more details about the use of this panel).

4.2.3 PACKWIN Operant Vogel Test Module (VT)

In the PACKWINVT, the only available box configuration available is the Vogel test box configuration.



Box configuration with virtual boxes is the same than with real boxes (please refer to the previous chapter 4.1.3.2. for more details about the use of this panel).

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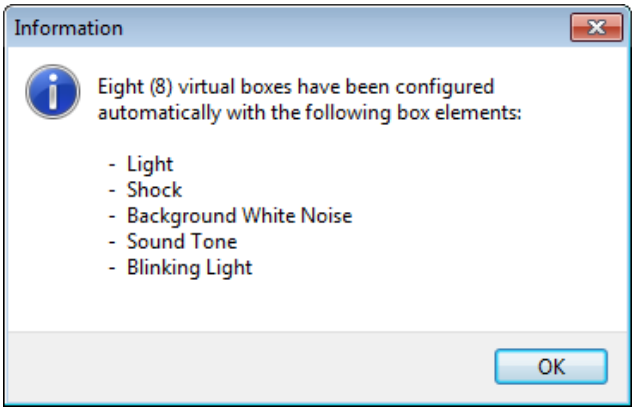
4.2.4 PACKWIN Freezing Module (CSFR)

In the Virtual boxes mode, the **Box Configuration** panel is directly shown for virtual boxes configuration.

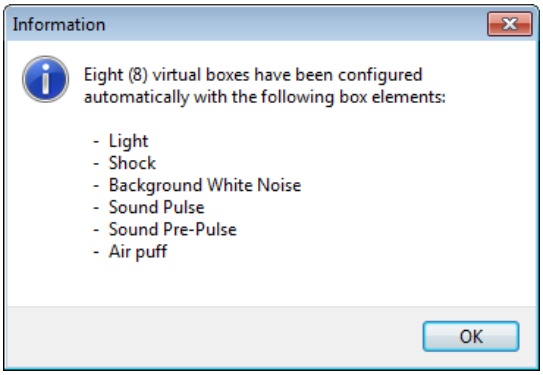
The FREEZING experiment module does not provide the **Box** Configuration panel. A predefined set of 8 boxes are automatically configured with the elements defined in the existing boxes configuration file ("jaulas.box").



4.2.5 PACKWIN Startle Module (CSST)

In the Virtual boxes mode, the **Box Configuration** panel is directly shown for virtual boxes configuration.

The STARTLE experiment module does not provide the **Box** Configuration panel. A predefined set of 8 boxes are automatically configured with the elements defined in the existing boxes configuration file ("jaulas.box").



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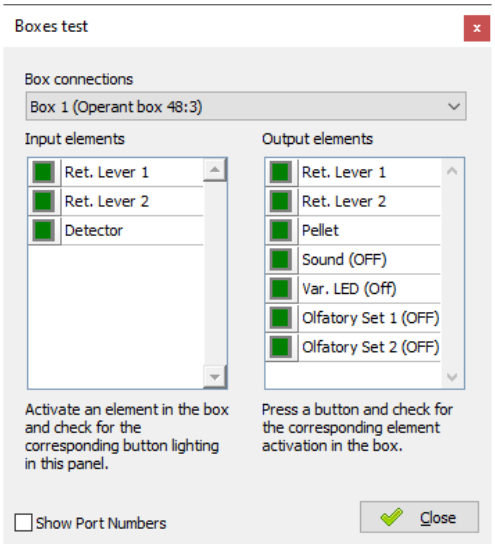
5 Boxes Test

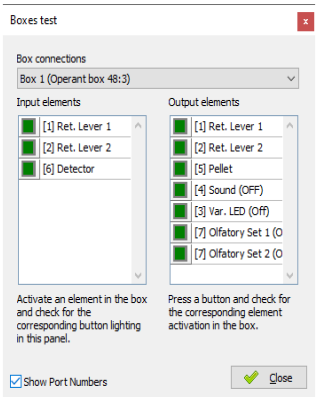
The **Boxes Test** option is available for checking for the operational status of all the input and output elements of the box directly from the software, before beginning the experiment. It is recommended to perform the box test each day before initiating the first experimental session.

This option is only available when working with real boxes. Please refer to the chapter 4.2. for more details on how to configure the system to work with real boxes.

- Press **Boxes Test** button:
- The **Boxes test** dialog is shown including the different elements configured in the particular kind of boxes detected. The BOX TEST panel provides a test button for both Input (responses) and Output (stimuli) elements of the boxes used for the experiment. The Input and Output elements shown depends on the box configuration purchased by the user.



- Use the **Box connections** section to select a box. One panel is available for each box.
- Test the input elements by activating the elements manually in the box and checking for corresponding button lighting in the **Input elements** section.
- Test the output elements by pressing the corresponding button in the **Output elements** section and checking for the element activation in the box.
- Repeat the process for each of the boxes connected to the computer.
- Mark the option "Show Port Numbers" to see the I/O port where the elements should be plugged in.



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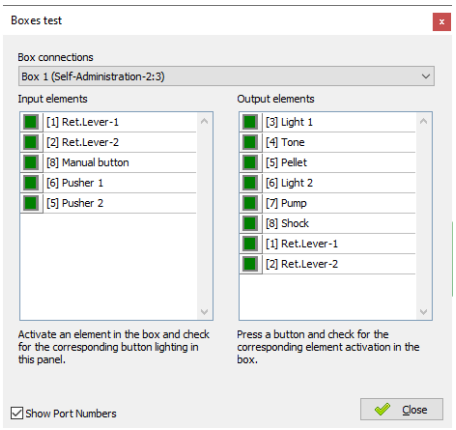
5.1 PACKWIN Operant Module (CS)

When working with the PACKWIN Operant module (PACKWINCS), the Input and Output elements shown depends on the box configuration purchased by the user. Below, is an example of a self-administration box.

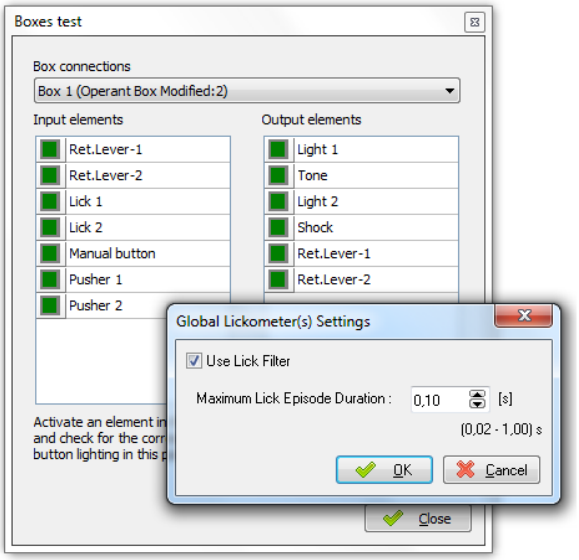


5.1.1 Case of operant boxes with Lick meter(s)

If the operant box includes one or several lick meters, the user is able to configure a theoretical duration for one lick. It may be used if the animals are doing long episodes of drinking by keeping "permanent" contact with the bottle spout.

This option is accessible by clicking on a Lick element.

A "Global Lickometer(s) Settings" panel appears.



If the option "Use Lick filter" is not checked, a lick will be strictly defined by an episode of contact with the bottle spout, whatever the duration is.

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If the option "Use Lick filter" is checked, a "Maximal Lick Episode Duration" has to be set by the user, in a range of 0.02 and 1 second. During the data acquisition, PACKWIN will divide the episodes of contact with the bottle spout having a duration higher than this value entered.

As an example, if this option is checked and the Maximal Lick Episode Duration is set at 0.1 second, an episode of contact of 0.55 second will be interpreted as 6 licks (5 licks of 0.1 second and 1 lick of 0.05 second).

The settings defined for this option is applied to all lick meter modules present in the operant cages.

If this option has been used for a session, it is specified in the column "Remarks" of the analysis reports, with the value defined as the Maximal Lick Episode Duration.

Note: This parameter has to be set before the data acquisition and is not modifiable afterward in sessions already acquired.

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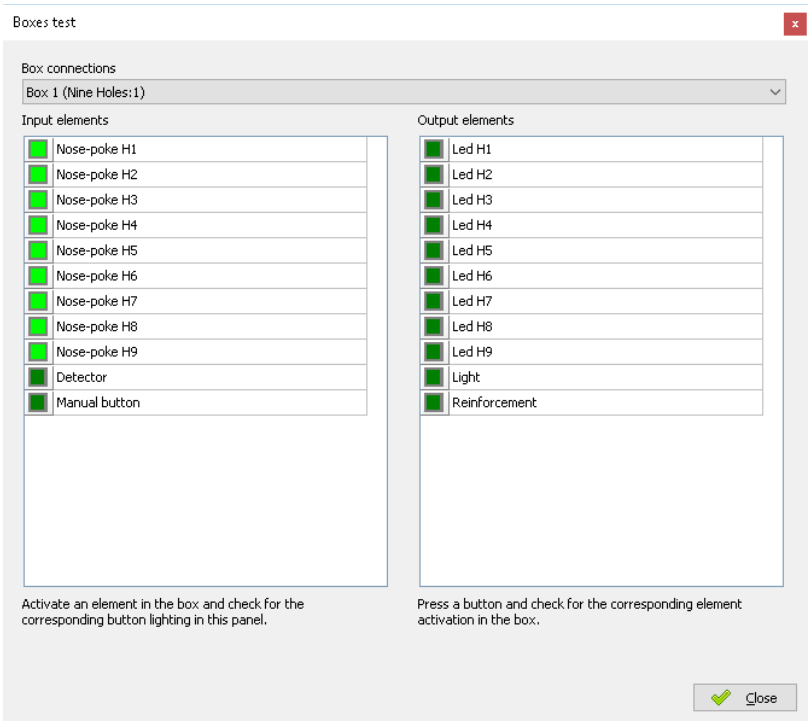
5.2 PACKWIN Operant Module (HO)

The BOX TEST panel for the PACKWIN-HO modules provides a test for both Input (responses) and Output (stimuli) elements. The Inputs and Output elements shown depends on the model of nine hole box purchased by the user. Below, is an example of a typical nine hole box.



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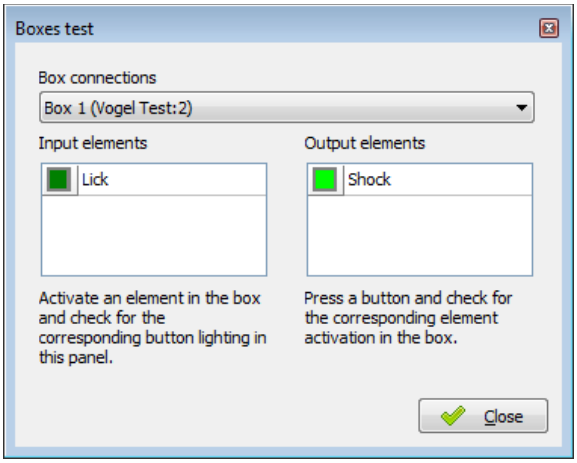
5.3 PACKWIN Operant Module (VT)

The BOX TEST panel for the PACKWIN-VT modules provides a test for both Input (responses) and Output (stimuli) elements. The Inputs and Output elements shown are the same for all Panlab Vogel test boxes (1 lickometer and 1 shock generator).



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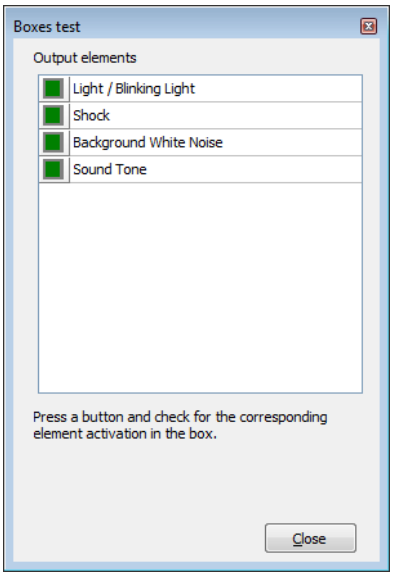
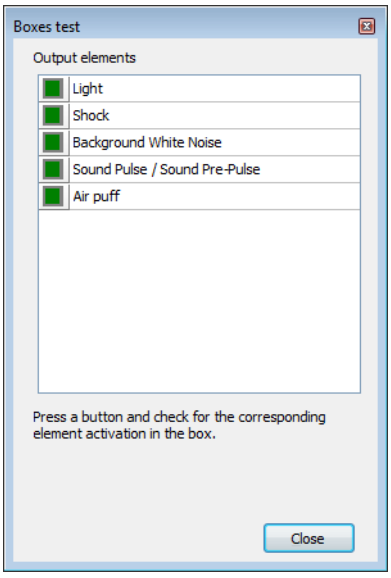
5.4 PACKWIN Freezing and Startle Modules (CSFR and CSST)

The BOX TEST panel for the PACKWIN-CSFR and PACKWIN-CSST modules only provides a test for the Output (stimuli) elements. The Input element (animal movement detected through the load cell) is tested directly by checking the movement of the galvanometer provided from the frontal panel of the LE111 control unit.

The Output elements shown are the same for all Fear conditioning boxes (see next figure).



The Output elements shown are the same for all startle boxes (see next figure).



For FREEZING and STARTLE boxes, the test operation is done for all the boxes at the same time given that the stimuli are provided and generated by a shared control unit (LE118 unit).

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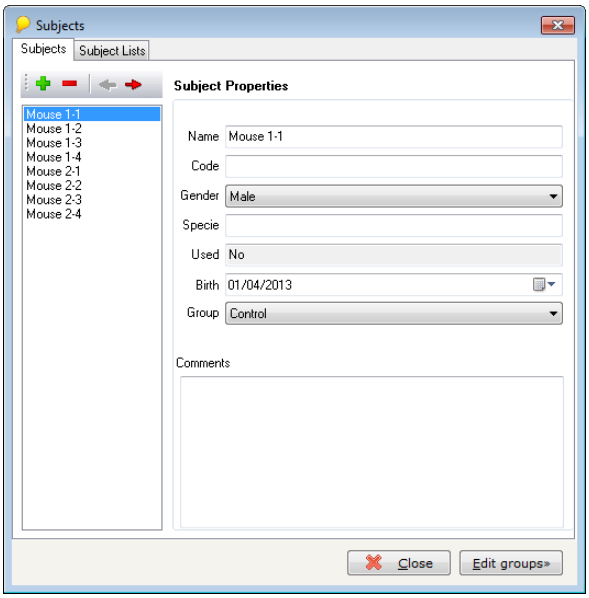
6 Subject Database

The PACKWIN **Subject** database collects all the characteristics of the subjects used in the experiments (name, gender, groups, registered sessions, etc.).

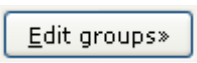
User-defined lists of subjects (**Subject List**) can also be created in order to facilitate the distribution of the subjects in the different experimental boxes before starting the experiment.

6.1 Subject Management

To access to the Subject database, press the **Subjects** button and stay with the Subjects tab activated.



- To add a new subject:
  - Press the button.
  - Enter the information of the subject (name, code, Gender etc.). Group 1 is given by default.
  - The added subject will be presented in alphabetical order in the left-section list.
- To delete a subject:
  - Select the subject to delete in the left-section list.
  - Press the button.
- To edit groups:
  - Press the **Edit groups** button to enlarge the configuration panel:



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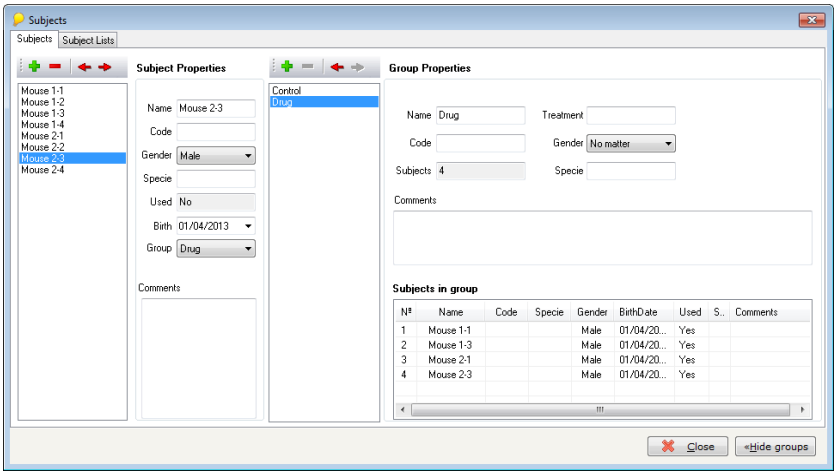

- The name and related information of the selected group can be modified using the corresponding available option in the **Group Properties** section.
- Press the button to add a new group. This new created group will be now available for selection in the **Group** list of the **Subjects Properties**.
- See the subject information currently included in the groups in the **Subjects in group** section.
- Press the button to delete a group (a group can only be deleted if it is not associated to any subject).
- Press the **Hide groups** button to hide the Group configuration panel.

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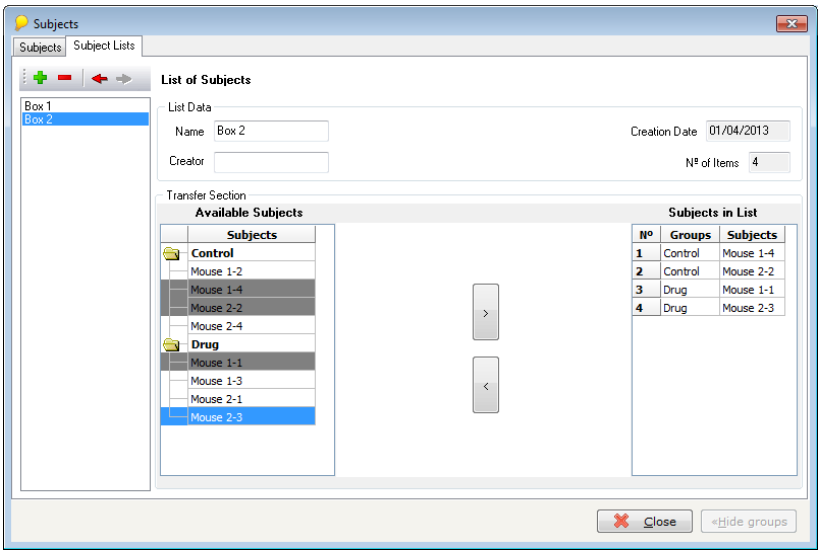
6.2 Subject Lists

Subject lists are commonly used during acquisition as they provide an easy way to select the subjects to run in the sessions.

For instance, in an experiment in which the session has to be repeated each day for each animal with subjects exposed in the same order to the experimental design, a convenient use of these lists is to configure a list of subjects for each box.

- To create lists of subjects:
    - Press the **Subject Lists** Tab for accessing to the Subject Lists panel configuration.



    - Press the button to add a new subject list.
    - Enter general data related to this list in the **List Data** section.
    - Select the subjects in the **Available Subjects** table (can be one by one selection or multiple selection by pressing the CTRL key).
    - Use the arrow buttons to insert or remove the selected subjects into/from the new list.

- To delete a subject list:
    - Select a subject list in the left panel.
    - Press the button to delete the selected list.

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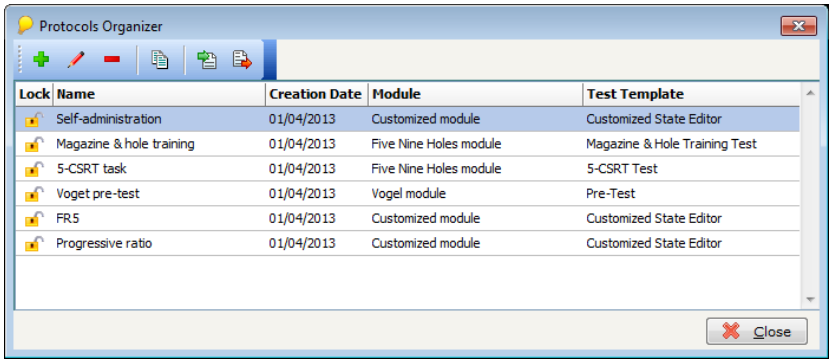
7 Experimental Protocol

7.1 The Protocol Organizer

PACKWIN provides a specific tool for managing the protocols contained in the current experiment file: editing, modification, information...



The **Protocols Organizer** window is composed of a protocol table and a tool bar.

7.1.1 Protocol Table

This table provides useful information about the protocols available in the experiment file. The following information is displayed:

- **Locked/unlocked**: indicates whether the protocol is locked or unlocked for editing. A new protocol remains unlocked until the data of a session have been saved. For traceability reasons, a locked protocol can be visualized but cannot been edited/modified. If the user would like to introduce a change for using the new modified protocol in futures sessions, the protocol has to be first copied. The copy of the protocol is then available for editing.

  A icon may appear in the **Lock** column indicating that the selected protocol is not suitable for acquisition (incomplete edits, unavailable module...).

- **Name**: indicates the name of the protocol given by the user.
- **Creation Date**: indicates the date of the creation of the protocol.
- **Module**: indicates the experimental module used for editing the protocol (nowadays, it can be the Operant customized, Five Nine Holes, Vogel, Startle or Freezing test modules).
- **Test Template**: indicates the test template used for creating the protocol.

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7.1.2 Tool bar

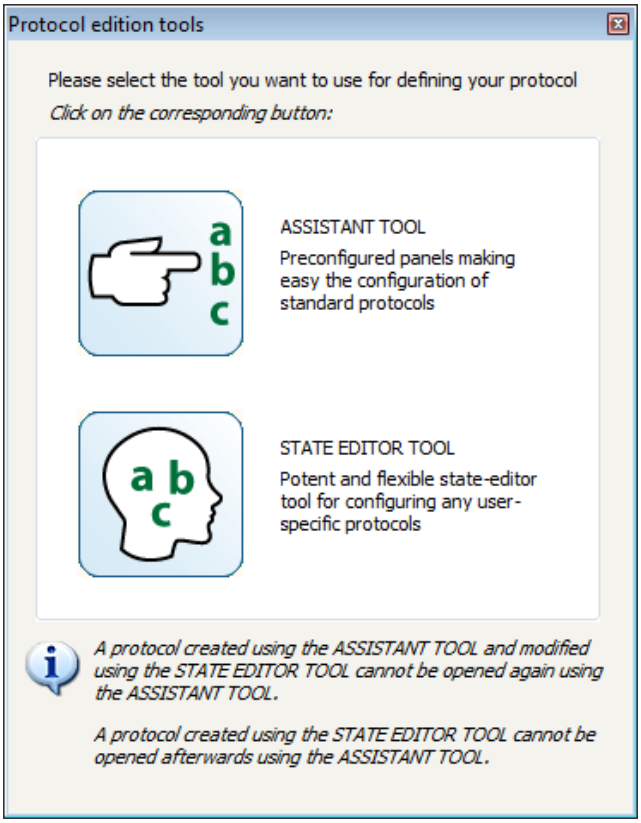
The tool bar contains buttons for creating, editing, deleting, copying, importing and exporting protocols.

7.1.3 Create a new protocol

To create a new protocol:

- Press the **New** button.

In the CS Module, PACKWIN offers two ways for creating and configuring a new protocol: **Assistant tool** and **State Editor** tool:



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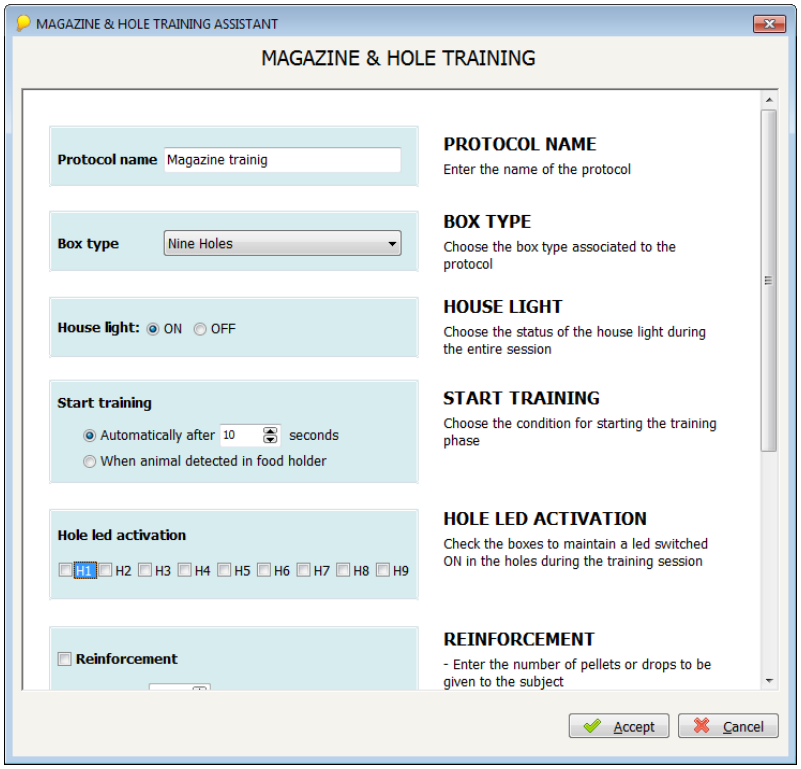
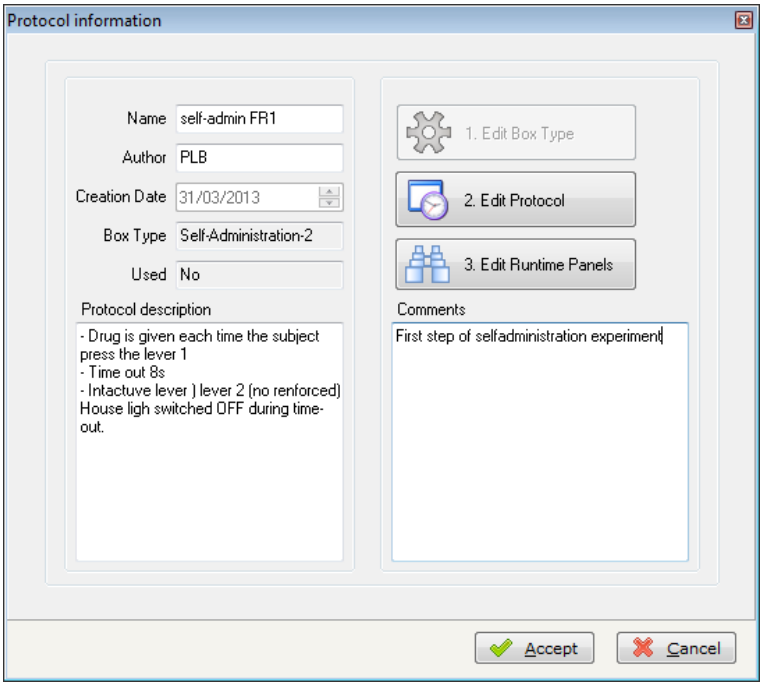
- The **Assistant tool** provides straightforward and predefined panels to facilitate the editing of standard protocols.



- The **State editor tool** provides optimal flexibility for configuring basic schedules for operant procedure as well as more specific and complex user-defined protocols.



A different protocol configuration tool will be available depending on the PACKWIN modules purchased.

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7.1.4 Edit a protocol

To edit a protocol:

- Select the protocol to be edited by clicking on one of the items of the protocol organizer table.
- Press the **Edit** button.

If the protocol was created using the Assistant tool, the application gives the opportunity to edit the protocol with the Assistant or with the State editor tool.

If the protocol was created using the State editor tool, it will be directly managed by the State editor tool.

7.1.5 Delete a protocol

To delete a protocol:

- Select the protocol to be edited by clicking on one of the items of the protocol organizer table.
- Press the **Delete** button.

Deleting a protocol will also **irreversibly delete all the sessions and corresponding data registered with the protocol**. After deletion, these sessions and data will be lost. In other terms they will not be available from the Analysis section panel anymore.

7.1.6 Copy a protocol

Sometimes, a protocol is very similar to another, but they differ in small details. To edit a new parameter, it can be helpful to make a copy of an existing protocol and start the changes from this copy.

To copy a protocol:

- Select the protocol to be edited by clicking on one of the items of the protocol organizer table.
- Press the **Copy** button. A new line will be added with the name of the original protocol with the prefix "Copy of…".

The copy of a protocol includes all the configurations defining the original protocol (assistant or state editor configurations, Run-Time panel, etc.) except related registered sessions.

Copying a locked protocol will unlock it for enabling editing.

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7.1.7 Export and Import a protocol

The Export/Import option is available for transferring protocols from one experiment file to another.

This option is particularly useful when the user aims to use the same protocol in different experiments without having to define it again in the new created experiment file.

- To export a protocol:
    - Open the experiment file containing the experiment file to be exported.
    - Select the protocol to be edited by clicking on one of the items of the protocol organizer table.
    - Press the **Export** button. Choose a destiny folder to which the exported protocol will be sent and accept.
    - The selected protocol will be exported with the *.pwp extension.
- To import the protocol in the destination experiment file:
    - Open the destination experiment file in which the user wants to transfer the exported protocol.
    - Press the **Import** button in the corresponding Protocol Organizer panel.
    - Select the protocol to be imported and accept.
    - The selected protocol will be imported with the prefix "Import of…".

Exporting/Importing process of a locked protocol will unlock it for enabling editing in the designatory experiment file.

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7.2 Edit a Protocol with the State Editor Tool

The PACKWINCS, PACKWINCSFR, and PACKWINCSST modules are the three PACKWIN modules using the state editor tool for editing the protocols that will be used during acquisition.

The Customized module provides the flexible and easy-to-use **State Editor Tool** for configuring the experimental protocols requested by the user.

The edit is based on a chain of state, in which the user defines the elements activated (light, sound, pellet dispenser, etc.) and the conditions to go to the next protocol state (after x time elapsed, after animal lever pressing, etc.).

The main differences between the PACKWINCS, PACKWINCSFR and PACKWINCSST modules are:

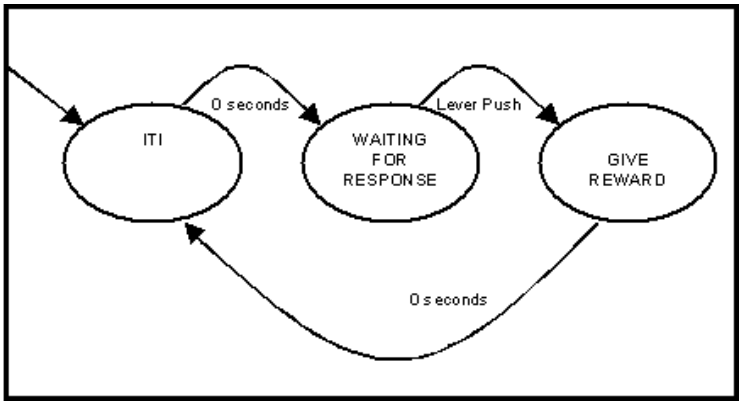
- Input/responses elements can only be used and recorded in the PACKWINCS modules. The PACKWINCSFR and PACKWINCSST modules only use output stimulus (light, sound, shock). The responses (immobility or startle responses) are automatically generated through the analysis of the signal provided by the weight transducers).
- The PACKWINCSFR and PACKWINCSST use specific panels for the configurations for the light and the sounds.

7.2.1 Protocol Structure

In the State Editor Tool, a protocol is a written procedure describing, in detail, the steps to conduct a controlled study (usually a preclinical study or clinical trial) to ensure the collection of valid data.

Protocols determine the structure of a PACKWIN experiment: Animals are set to work inside protocols, experimental chambers run according to protocols, and measurements differ from protocol to protocol.

Structurally, a protocol is a set of states. Every state has connections to other states (state-change conditions) and events taking place inside (initializations). The whole set of states and connections are called Chain of Finite States.



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In this manual, examples of the mentioned protocols are often detailed. The terms used can be either very specific (pellets, light) or more theoretic (reinforcement). The application of such terms will depend on the context. In the following, we will use equivalently:

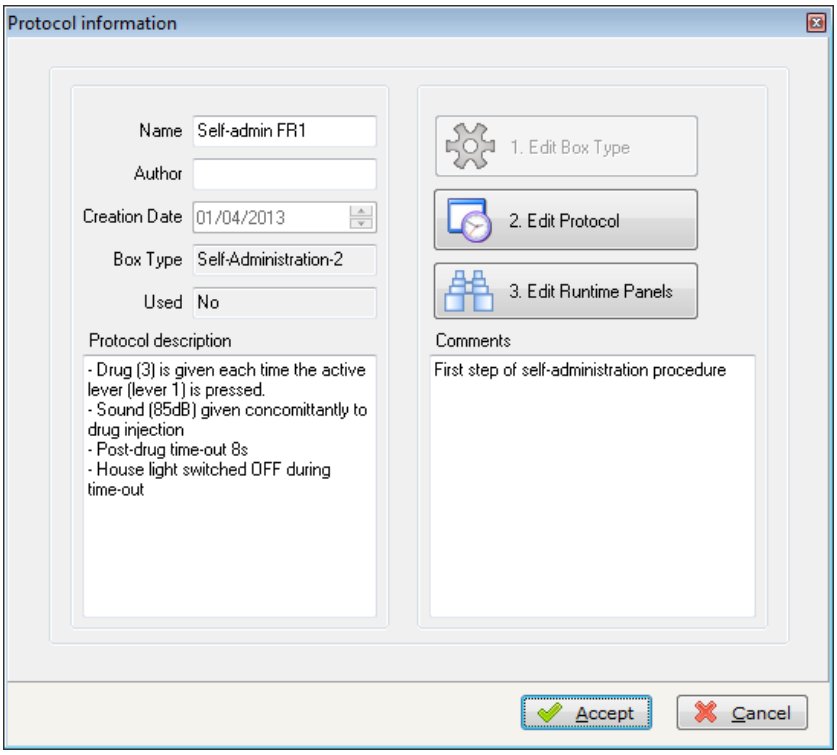
- Positive Reinforcement (or Reward): Pellets, Liquids, Drugs, Cerebral Stimulus, etc
- Negative Reinforcement: Shock, etc
- Stimulus: Light, Sound, etc
- Response: Lever pressing, reward magazine visit, nose poke detection etc.

7.2.2 The Protocol information panel

When the State Editor tool option is chosen a Protocol information panel is opened giving access to 2 sections:

- A section for entering general information about the current protocol.
- A section for editing the protocol.

The editing process is divided in 3 steps: associating the protocol with a given box type, editing the sequence of the protocol (states) and editing the associated Run-Time panel.



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7.2.3 Enter protocol general information

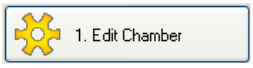
Use the available panel for entering the protocol general information:

- **Name** (required): user-defined name of the protocol. The same name cannot be repeated in the same Protocol Organizer table.
- **Author** (optional): enter the name of the editor.
- **Protocol description** (optional): here the user can enter a description of the protocol with words and sentences. This section provides a general rapid view of the protocol structure without having to examine the details of the editor sections provided into the Edit Protocol section. The content of the Protocol description section can also be visualized for the acquisition panel.
- **Comments** (optional): additional section for adding extra user-defined information.

The Creation Date, Box type, and Used sections are automatically filled by the application.

- **Creation Date**: date of the creation of the protocol. A protocol is considered as created when, at least, the following suite of operation has been followed: (1) a name has been chosen, (2) a Box type has been related to the protocol and (3) the Protocol information panel has been closed using the Accept button.
- **Box type**: this information is shown once a Box type has been related to the protocol.
- **Used**: this section indicates whether the current protocol is associated with registered sessions (**Yes**) or not (**No**).
-

7.2.4 Choosing a Box type



In The CS Module, a protocol is linked to a specific type of box (chamber). Therefore, before editing the aspect of the protocol, the appropriate box type must be chosen.

To choose a Box type, press the Edit Chamber button and then select the desired box type from the available **Box type** list (Skinner box, Self-administration, nine-holes, etc.).

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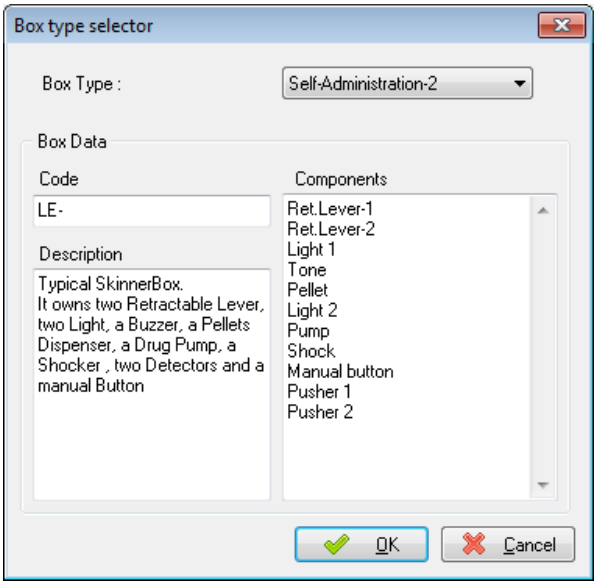

The list will only show the box types purchased by the user. Each box type contains the specific elements of the box that were chosen by the user during the sales process.

The **Editor for Box** panel also shows the following not-editable sections:

- **Code**: sales reference for each box type (when it exists).
- **Description**: Internal description of the box together with the description of the elements contained in it.
- **Components**: List of the elements defining the Box type.

  Please, make sure all the elements installed in your experimental box appear in the list of elements shown in the **Components** section of this panel.

  If not, the PACKWIN functions can be compromised. Please contact your sales representative so that we may provide you a new Box type choice option. It specially occurs when using the PACKWIN software OPERANT modules and when the configuration of the different elements of the box (lever, light, feeder, sound, pump, etc.) are changed by modifying them or adding new ones. Please refer to the chapter 2.6.1. for more details about the box configuration files.

When the box is selected, the structure of the protocol is ready to be edited. By clicking on the Edit Protocol button, the Protocol State Editor is opened.

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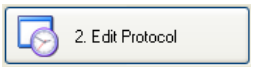
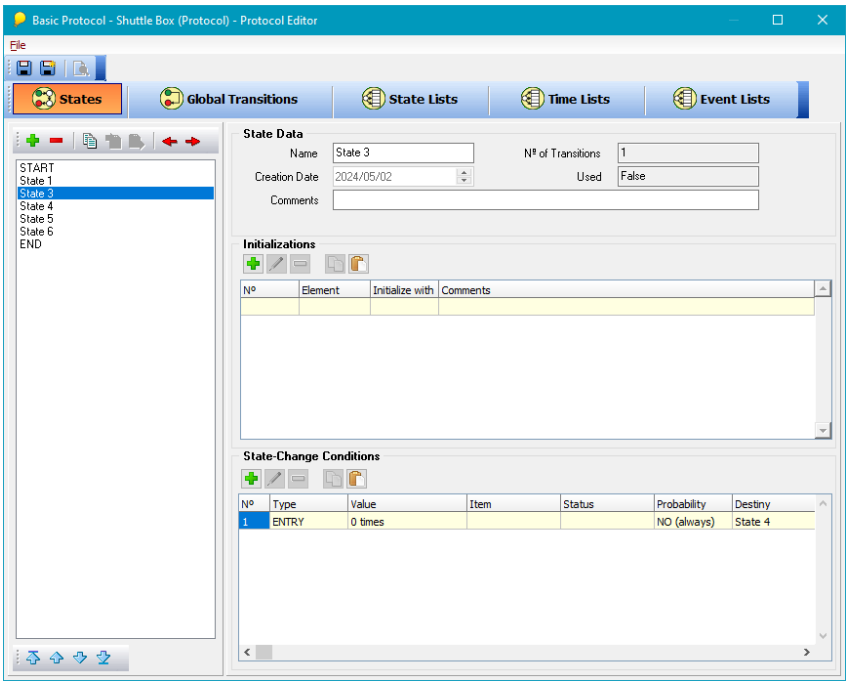


7.2.5 Editing the Protocol

The Protocol Editor allows the experimenter to build protocols, i.e., choose the number of states, transitions between them, the events taking place in every state, etc.



The structure of a protocol in PACKWIN is as follows:

- An initial state called START.
- A chain of finite states.
- A final state called END.
- Global Transitions.
- Lists of States.
- Time Lists.
- Event Lists.

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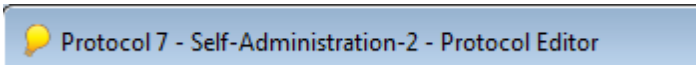
7.2.5.1 The Protocol Editor main panel

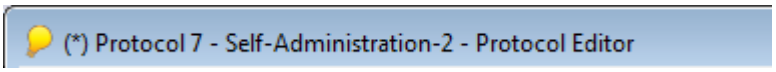
The **Protocol Editor** main panel is shown with the following elements:

- **Title bar**



The title bar shows the name of the current protocol and the name of the associated box type.

The name of the protocol is preceded by a (*) when changes have been made in the protocol that have not been saved yet.



- **Main menu bar**

The main menu bar contains the following:

Save: saving the protocol with the current name.

Save as: saving the protocol with another user-selected name.

Close: closing the Protocol windows for returning to the Protocol Organizer panel.

- **Protocol Editor Assistant bar**

The **Protocol Editor Assistant bar** has been designed to give the experimenter a quick way to access to the main editing sections.

This bar is designed in a way that only the currently allowed sections are active.

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7.2.5.2 The States section

A State is the representation of a protocol at a given time.

Before setting the software, it is recommended to the user to have clearly in mind the Chain of States defining the protocol, as well as all the parameters that will define each state (e.g. what will happen when the system will enter into the state and the conditions allowing the change to next state).

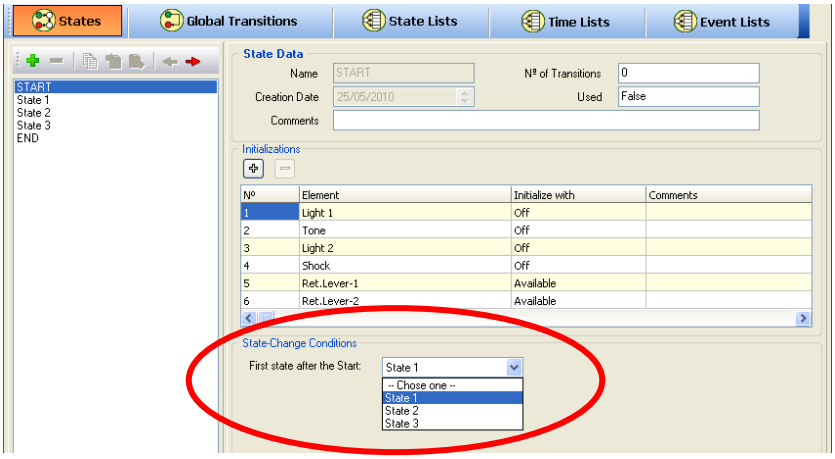
The States section allows defining the different states of the protocol and their connections (state-change conditions and initializations). These operations will define the main structure (body) of the protocol.

In PACKWIN, the States START and END are always present. The missing states (1 to n) have to be created.

7.2.5.3 The START and END states

Every protocol begins in the START state and stops when it arrives to the END state.

The START state is the first state in every protocol. The starting configuration of the chamber is set here, i.e. every element is initialised here. After this, the protocol goes to the state pointed by the State-Change Conditions in **First state after the Start** (see next figure).



Note: To be allowed to choose a state as First state after the Start, the Chain of states of the experiment has to be first created.

The END state is the last state in every protocol. To finish a session the end state must be reached, so any condition to exit the protocol must point to the END state.

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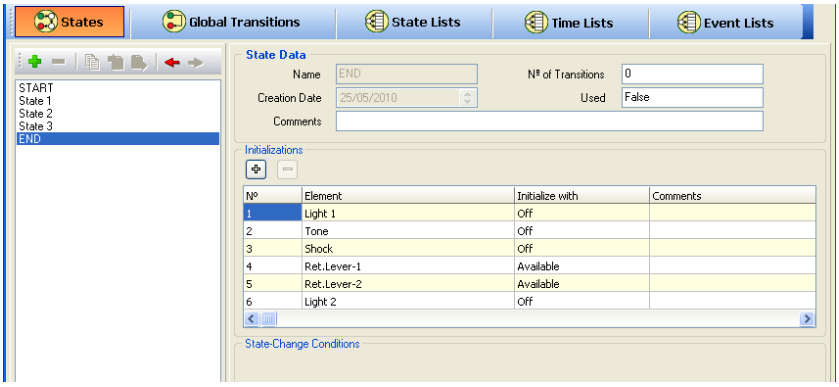
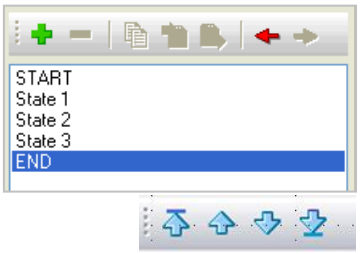


It is important to deactivate in the END state all the elements that have been used in the protocol.



7.2.6 Editing the chain of state

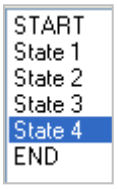
7.2.6.1 States management

All the created State can be visualized in the list section placed on the left part of the panel.

This section is provided with a tool bar for creating and deleting the State created.

ADD NEW STATES

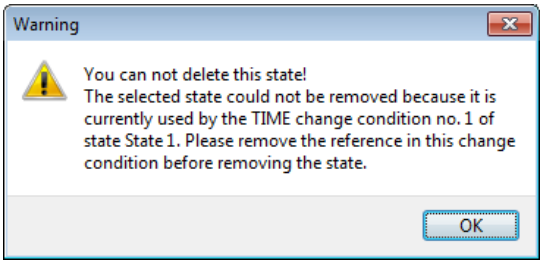
To add a new state, click on the **Add** State button located on the top of the states list. In the next example, 4 states have been created.



DELETE A STATE

Press the **Delete** button for deleting the selected state from the list.

If the selected state is implicated as **Destiny** in one of the state-change conditions, it cannot be deleted. The application will open the message shown bellow.



Please first unselect the state in all the indicated state-change conditions and try again.

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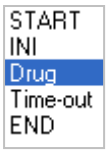
COPY A STATE

Another option is to make a copy of an existing state. Similarly to the case of protocols, sometimes two states can be very similar and instead of building one from scratch, it is easier to make a copy of an existing one. To do so, just select the state to be copied and click on the **Copy** State button.

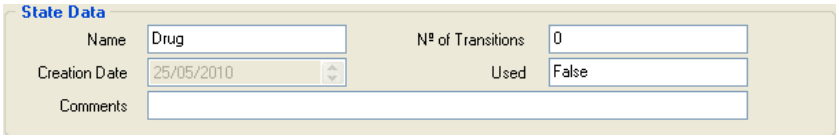
CHANGE THE ORDER OF A STATE

With the three buttons at the end of the left panel, it is possible to move the selected state at the top or bottom of the list ( ) or on position up or down in the list ( ).

7.2.7 The State data section



The State data section allows entering user-defined name for each state and adding comments.



This section also shows the following non-editable items:

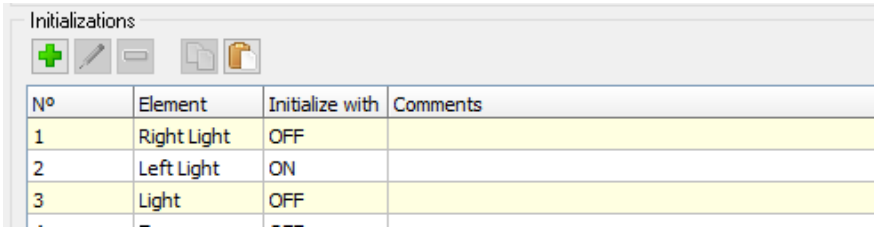
Nº of Transitions: number of state transitions involving the selected State.

Creation date: creation date of the selected state.

Used: whether the selected State is used.

7.2.7.1 Initializations

Initializations are the events (light, sound, shock etc.) that will occur when the protocol enters into selected state.



To add a new element initialization (activation or deactivation), press the (**+**) button. The Edit element initialization dialog box will be shown.

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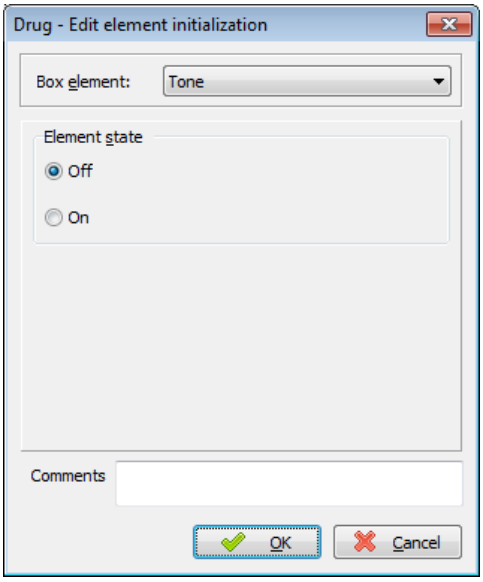
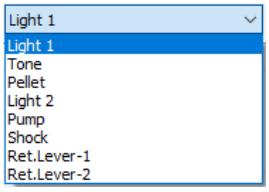


Chose the element to be activated/deactivated in the list available from the **Box element** section.

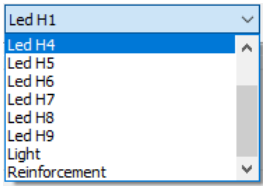
The Elements to be chosen in the Initialization section are all output-type elements and will depend on the Box type selected in the previous step.

Here some examples:

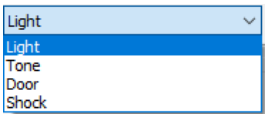
Operant box



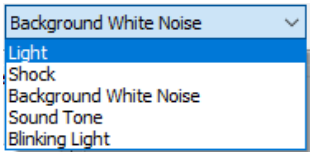
5/9 holes



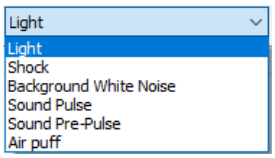
Shuttle box



Fear conditioning box



Startle box



Choose the state/status of Element in the **Element state** section.

The state or the status of each element is specific of each output element and can be of the following categories:

(A**) On/Off** elements – This status is mainly related to the activation and deactivation of the stimulus (Light, sound…) or negative reinforcement (shock…) in the current state. An on/off element stays in the selected status until this status is changed in another state.

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(B) **Not Available/Available** (retractable lever) – this status is mainly related to the status of availability of the retractable lever for the animal.

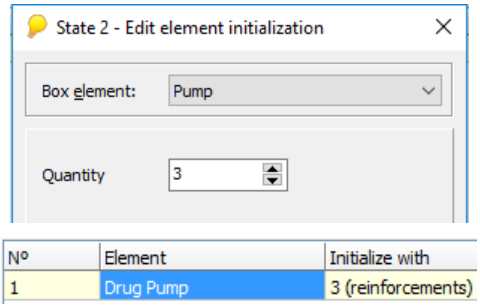
(C) **Closed/Open** (doors…) – this status is mainly related to the status of the guillotine doors in the shuttle box.

(D) Activated with a user-defined **quantity** (pellet dispenser, liquid dispenser…) – in those cases, the user has to enter a quantity of reinforcement to be given: number of pellet, number of liquid drops…

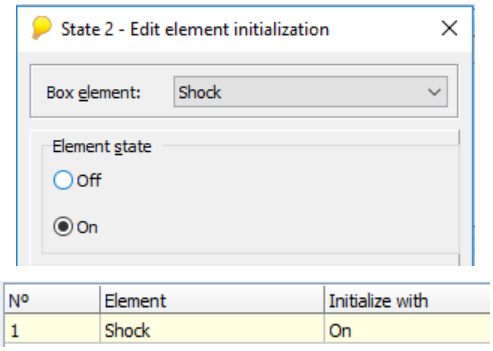
(E) Activated during a user defined period of **time** (drug pump…) - in that cases, the user has to indicate during how many time the element will be activated: duration of activation of the drug pump for instance…

Specific cases:

Drug pump in PACKWIN Operant modules: PACKWIN asks for the Quantity. In that case Quantity refers to the duration of the pump activation expressed in seconds. If the drug injection lasts 5 s, the value 5 (reinforcements) has to be set.



Shock in PACKWINCSFR Fear Conditioning module: shock duration is set using the On/Off settings.



Shock in PACKWINCSST Startle modules: PACKWIN asks for the Quantity. In that case Quantity refers to the duration of the shock activation expressed in milliseconds. If the shock lasts 2 s, the value 2000 (reinforcements) has to be set.

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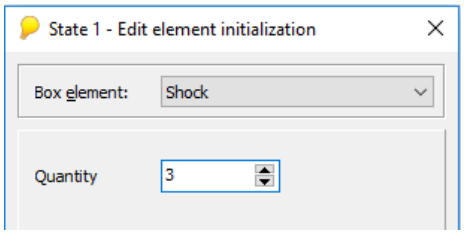
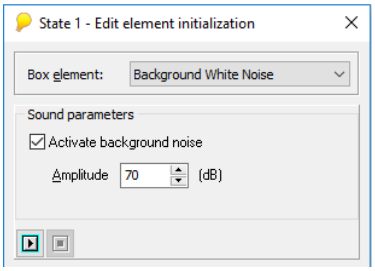


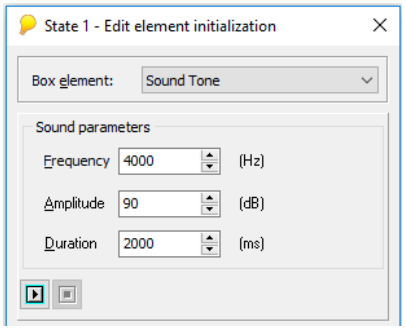
Nº	Element	Initialize with
1	Shock	3 (reinforcements)

Background noise in the PACKWINCSFR Fear Conditioning and PACKWINCSST Startle modules: the Background noise is an ON/OFF stimulus that also offers the user the possibility to set the Amplitude of the white noise. A Play button is available to test the sound with a sound meter.



Nº	Element	Initialize with
1	Background White Noise	White Noise ON (70) dB

Sound Tone in the PACKWINCSFR Fear Conditioning, the panel offers the user the possibility to set the Duration of the stimulus, as well as Frequency, Amplitude. A Play button is available to test the sound with a sound meter.



Nº	Element	Initialize with
1	Sound Tone	Freq (4000) dB (90) Time (2000)

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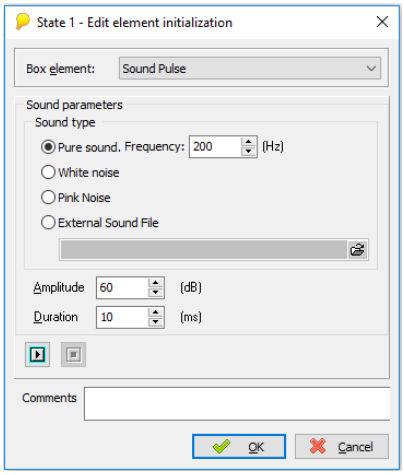
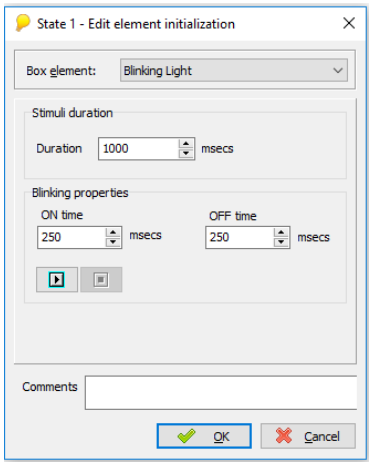
Sound Pulse, Sound Prepulse in the PACKWINCSST Startle module: the panel offers the user the possibility to set the Type of the sound as well as Amplitude and Duration. A Play button is available to test the sound with a sound meter.



An additional option allows the user to select the sound from an external "*.wav" file. The Amplitude of the sound would be the one set in the wav file. The Duration would be the one set in the PACKWIN panel. If the duration of the wav file is shorter than the Duration set in PACKWIN, it will be played in loop (repeat).

Blinking Light in the PACKWINCSFR Fear Conditioning and PACKWINCSST Startle modules: the panel offers the user the possibility to set the Duration of the stimulus, as well as the Blinking properties. A Play button is available to test the light pattern.



Nº	Element	Initialize with
1	Blinking Light	Blinking Light ON (250 / 250 msec), 1000 msec

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Setting a lever as **Available** or **Not available** is only useful when retractable levers are used. If the levers used are not retractable, selecting one of these options will not have any effect.

When the elements from the categories A, B and C are set in an user-defined status in a state (On, Closed...), they will remain in that status until the protocol receives the instruction for changing their status (Off, Open...) in another user-defined state, and vice versa. So, the duration of the activation of an element may depend on the duration of the state in which it has been activated.

The duration of the activation of the elements of the D and E categories are independent of the duration of the states in which they have been activated.

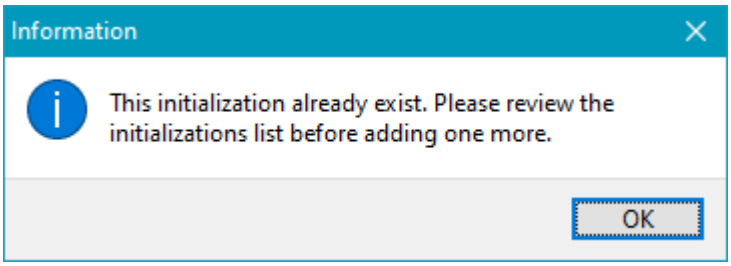
More than one Initialization can be entered into one state.

To delete an initialization (activation or deactivation), select the corresponding line in the Initialization table and press the (-) button.

Click on this icon to edit the initialization selected.

Click on this icon to copy an initialization in one state and paste it in another state.

Each state cannot contain the same initialization twice: the following warning appears when trying to paste an initialization in a state that already contains it:



7.2.7.2 State-change conditions

Conditions (also called as transitions) point to a new state, since every time a condition is fulfilled, a new state is reached. In other terms, the State-change condition edits how and when the current state will finish, and which state will follow.

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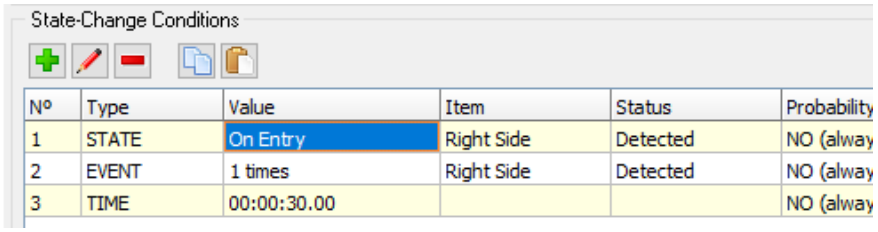
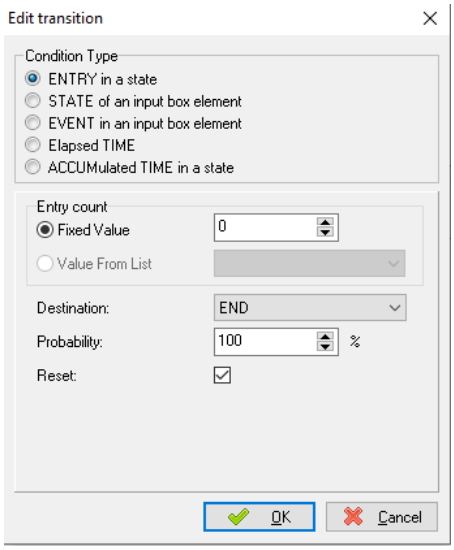


Click on this icon to edit the state-change condition selected.

Click on this icon to copy and paste a state-change condition and paste it in the same or another state.

To add or delete a change condition, use the (**+**) and (**-**) buttons at the top of the grid, respectively.

When the **+** button is pressed, the user is asked to choose the condition type in the Edit transition dialog box.



In this panel, 5 steps of configuration have to be followed:

(1) Selection of the Condition

(2) Value of the Condition

(3) Destination

(4) Probability

(5) Reset function

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(1) (2) Selection and value of the Condition

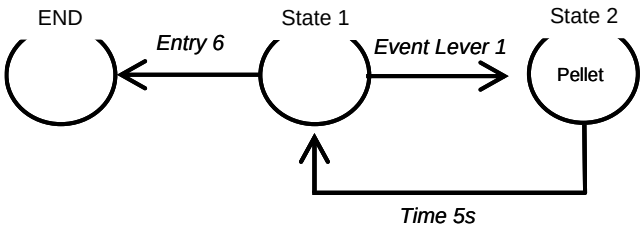
Conditions can be set upon four different options.

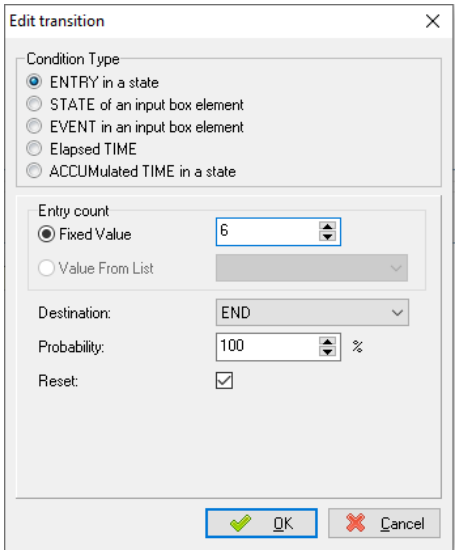
• ENTRY in a state:

The Entry in a state condition is used when the protocol enters in the next state after the current state has been executed/performed/repeated for a specified number of times.

Example:

When the subject presses the Lever 1 in the State 1, a pellet is given in the State 2. After 5s, the system switches automatically to the state 1 where the subject is allowed to press the Lever 1 again to receive a pellet. The cycle State 1-State 2 finishes when the subject has received 5 pellets.





In the state 1 configuration, add a new State-Change Condition. Select the ENTRY condition.

The **Entry count** field Value represents the number of times the protocol has to enter in the current state for fulfilling the condition and go to the next state (here, 6 times).

Select the END state as Destination and press **Ok**.

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Nº	Type	Value	Item	Status	Probability	Destiny	Reset
1	ENTRY	6 times			NO (always)	END	☑

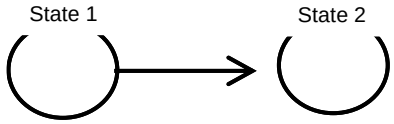
On the 1st, 2nd, 3rd, 4th and 5th entry in the state 1, the subject will be allowed to press the Lever 1 to obtain a pellet. On the 6th entry in the state 1 (i.e., after receiving 5 pellets), the experiment will be ended.

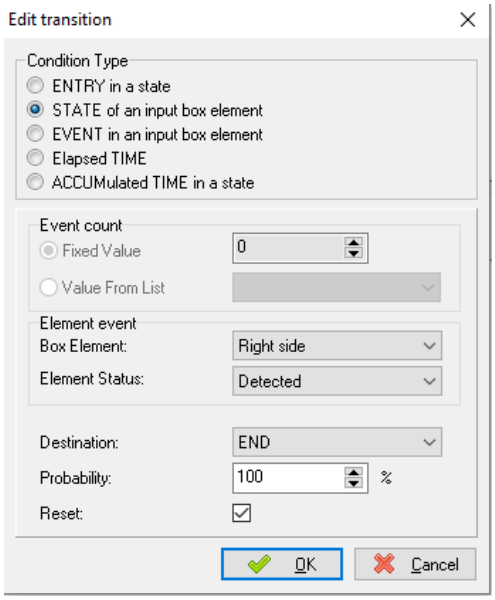
• STATE of an input element of the box

The STATE condition is used to move to another state depending on the status of an input element within the box.

Example:

Upon entering in State 1, if the animal is detected in the Right Side, the protocol must go to State 2.





The **Event count** field is disabled in this case.

In the **Element event** section choose the input element (Right Side) and its status (Detected). Set the state END as the destination state and press **OK**.

Nº	Type	Value	Item	Status	Probability	Destiny	Reset
1	STATE	On Entry	Right side	Detected	NO (always)	END	☑

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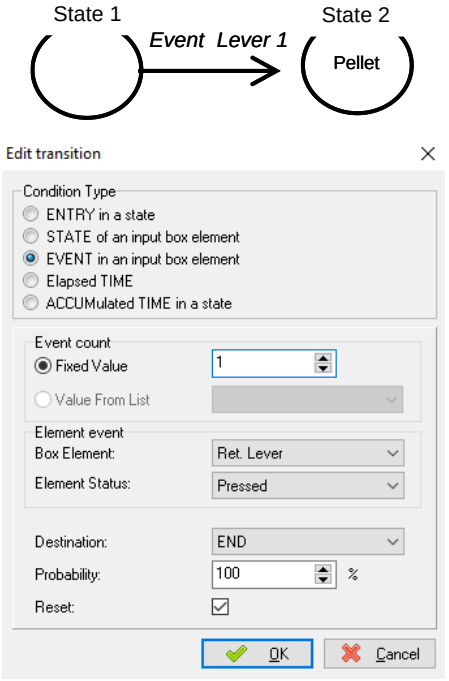
• EVENT in an input element of the box

The Event condition is used when the protocol goes to the next state when an event occurs in the experimental box (pressing a lever or un-pressing, food magazine visit, nose poke detection…).

Example:

The state 1 finishes after the subject activates one of the input elements of the box (e.g., lever, nose-spoke, food holder pusher etc.) and then the protocol goes to the State 2. In the next example, the subject receives a pellet when the Lever 1 is pressed.



Choose the condition type EVENT in an input box element.

The **Event count** field Value represents the number of times the subject has to act on the input element to fulfil the condition (in the next example, the subject has to press the lever 1 time to obtain a pellet; it represents a Fixed Ratio 1-FR1). Here, a **Fixed value** is chosen, but an Event list can be used allowing the configuration of Variable Ratio (**Value From List**, please refer to the chapter 7.2.6 for more details).

In the **Element event** section choose the input element (Ret.Lever-1) and its status (Pressed). Set the state END as the destination state and press **OK**.

Nº	Type	Value	Item	Status	Probability	Destiny	Reset
1	EVENT	1 times	Ret. Lever	Pressed	NO (always)	END	☑

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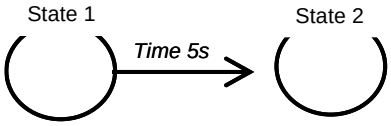
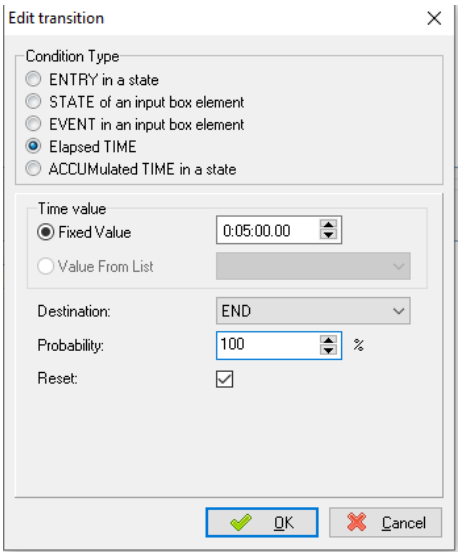
• Elapsed TIME

The Elapsed Time condition is used when the protocol goes to the next state after a user-defined period of time.

Example:

The state 1 finishes after a defined time (5 seconds in the next example) and then go to the State 2.





Select **Elapsed TIME** as the condition Type.

In the Time value field, enter the time value (hh:mm:ss.oo) that will define the duration of the current state. Here, a **Fixed value** is chosen but a Time list can be used allowing the configuration of elapsed time conditions with variable value (**Value From List,** please refer to the chapter 7.2.6 for more details).

Enter the Destination state and press **OK**.

Nº	Type	Value	Item	Status	Probability	Destiny	Reset
1	TIME	00:05:00.00			NO (always)	END	☑

• Accumulated Time in a state

The Accumulated Time in a state condition is used when the protocol goes to the next state after the protocol has been in the current state for a user-defined accumulated time.

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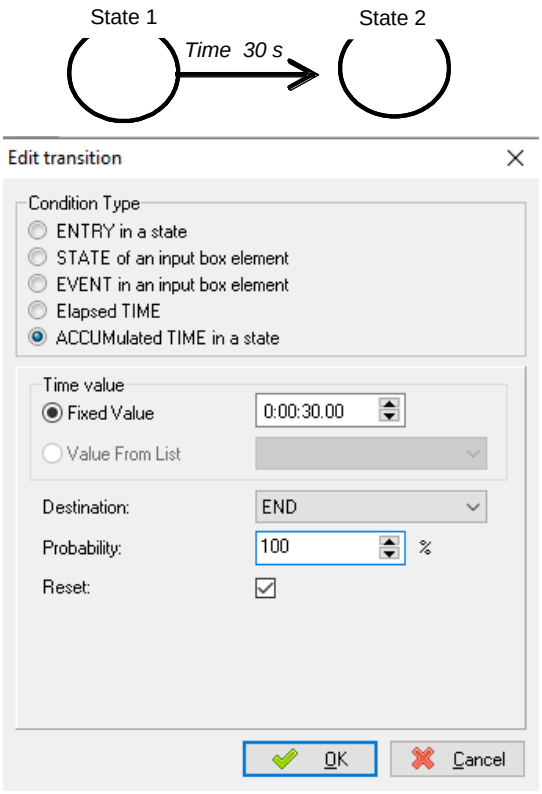
Example:

The State 1 finishes and then go to the State 2 when the protocol has an accumulated time of 30 seconds in the State 1.



Select the **Accumulated TIME** condition

Enter the desired amount of time in the **Time value** section (hh:mm:ss.oo). Here, a **Fixed value** is chosen but a Time list can be used allowing the configuration of accumulated time conditions with variable value (**Value From List,** please refer to the chapter 7.2.6 for more details).

Chose the destination state and press **OK**.

Nº	Type	Value	Item	Status	Probability	Destiny	Reset
1	ACCUM_TIME	00:00:30.0			NO (always)	END	☑

(1) Destination

Destiny is the name of the already existing state that the condition points to. Besides the normal states, there are two special cases: previous state and lists of states. The previous state is the one from which the session got in the present state.

Lists of states are detailed in the chapter 7.2.5.7.

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The START state cannot be targeted by any state-change condition.

(2) Probability

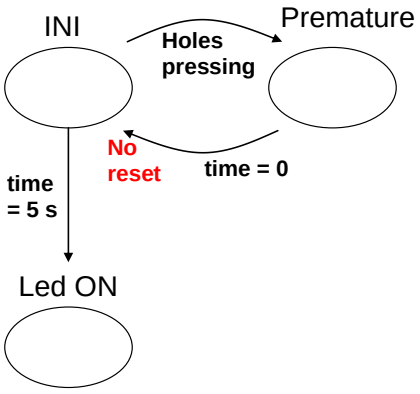
Alternatively, some conditions can have probability, so before the new state is reached, this probability is checked. The Probability field defines the probability of changing of state once the condition has been fulfilled.

(3) Reset function

The transitions to the destiny state can be chosen to be with the Reset mode (**Reset** checked) or the No reset mode (**Reset** Unchecked).

The checked mode is set by default and the unchecked mode in generally used when the user aims to reference back and forth from a specific state without resetting the time and event counter.

As an example, the **unchecked Reset** function can be used in the 5-choice serial reaction time task for counting the number of premature responses made during the INI. In that case, the INI period lasts 5 s and we don't want its time counter reset to zero each time the animal makes a premature response.



Here, a summary of the **Reset** and **No reset** mode effects on the Destination state:

The **Reset** mode (by-default mode):

- Checking of the status of the initializations.
- Resetting of the Time change-condition counters.
- Resetting of the Event change condition counters.

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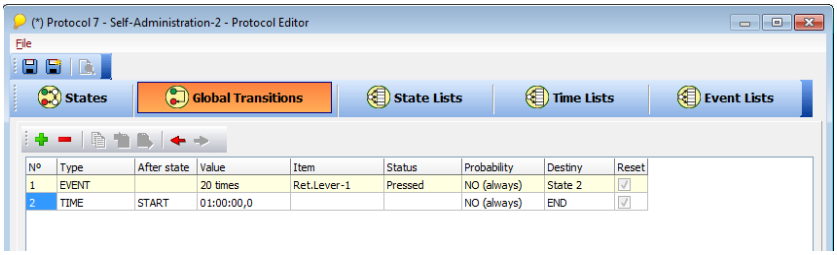
The **No reset** mode (= reset case unchecked):

- The status of the initializations is not checked and updated.
- The Time change-condition counters are not reset.
- The Event change condition counters are not reset.

7.2.8 Global Transitions section

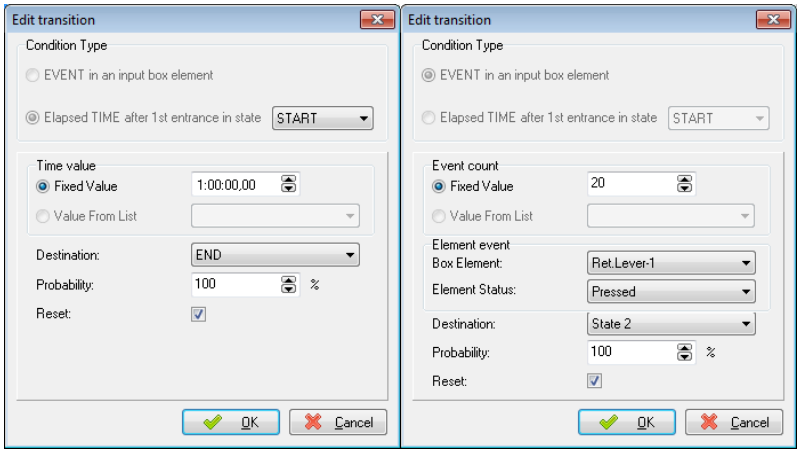
Global Transitions are conditions to change state that are checked independently of the currently active state for the protocol. These conditions are similar to the state-change conditions; the main difference is that they are not linked to a particular state, but to the protocol itself. Therefore, they are checked every time, no matter in which state the execution is at the present moment.



Use the corresponding (+) and (-) buttons to Add or Delete a global transition.

To define Global transitions, press the Global Transitions button and edit them as previously seen with state-change conditions. Similarly, to the state-change conditions, global transitions can be of two types: Time or Event.

A reference protocol state can be selected in the case of global time transitions. The global transition will be activated once the protocol enters in this state. The default is the START state which means the transition is active during the whole protocol execution.



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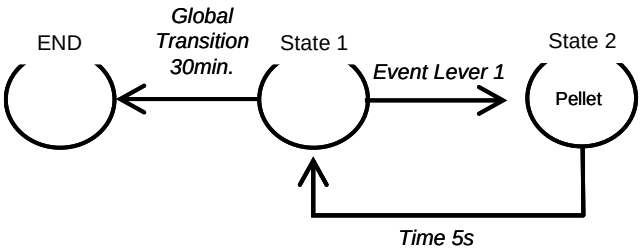
7.2.8.1 Elapsed Time condition

As an example, the protocol has to stop after 30 minutes training. The time spent in the session will be checked continuously (no matter which state the session is in) until the condition is fulfilled.



7.2.8.2 Event condition

As an example, the protocol has to stop when an animal received 30 intravenous drug injections (associated with the Lever 1 pressing). The number of lever 1 pressing made by the animal in the session will be checked continuously (no matter which state the session is in) until the condition is fulfilled.

Note: More than one global transitions can be selected in one session.

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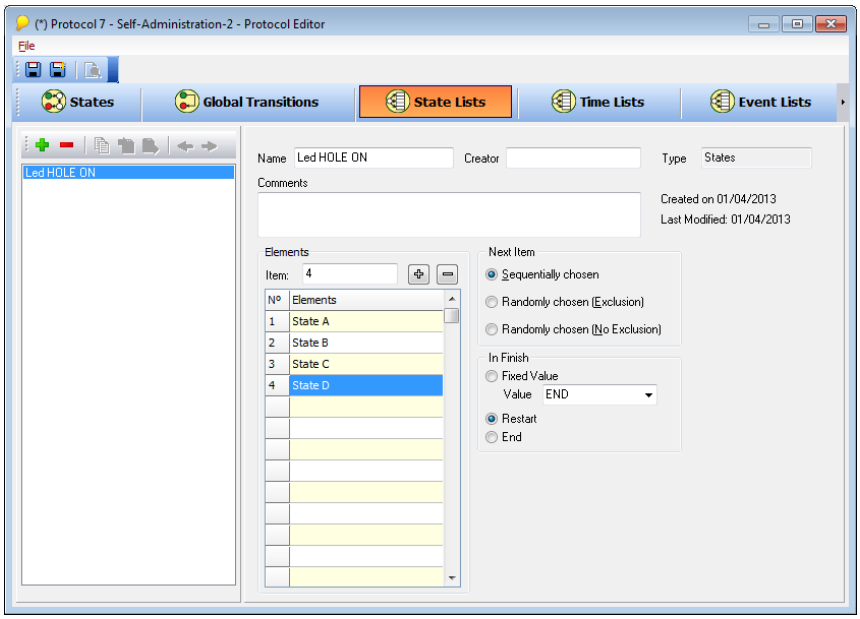
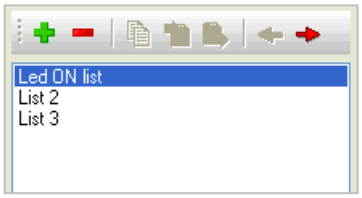
7.2.9 Edit a list of states

In some protocols, the upcoming state cannot be foretold. It is the case of random selection of the next state. Example: consider a Nine Holes protocol. A possible protocol could require that the hole were randomly selected but, in order to avoid biases, the same hole can't be selected more than three consecutive times. Building that protocol could be very complicated and need a big number of states. With a list of states, however, it can be easily done in PACKWIN.

Other times, different sequences should be joint into one protocol. In these cases, PACKWIN uses Lists of States.

A list of states is a set of states (state A, state B, state C...) in which every state can be chosen as a target for a state-change condition or a global condition.





All the created State Lists can be visualized in the list section placed on the left part of the panel.

This section is provided with a tool bar for creating and deleting the State lists created.

7.2.9.1 Add new State List

To Add a new list, click on the **Add** button located on the top of the states list.

7.2.9.2 Delete a State List

Press the **Delete** button and accept the confirmation message for deleting the selected list.

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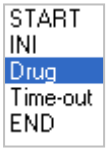
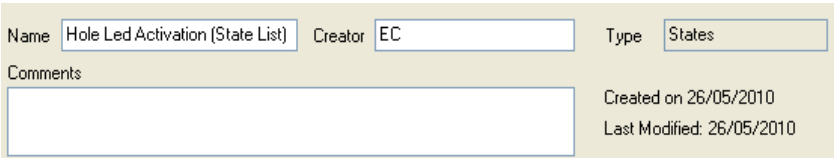
Note: If the deleted State list was already used in one of the state-change condition defined into the States section, do not forgot to change or eliminate these conditions in the corresponding state.

7.2.9.3 Edit the State data section



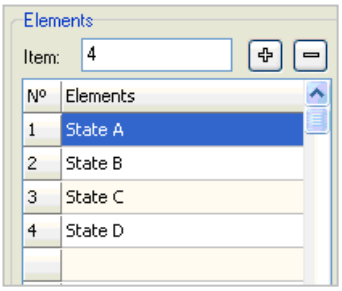
The State list data section allows entering user-defined name for each state, the name of the creator and also adding comments.



This section also shows the following not-editable items:

- **Type**: the type of the list (it can be a States, Event or Time list)
- **Creation date**: creation date of the selected state list.
- **Last Modified**: date of the last modification.

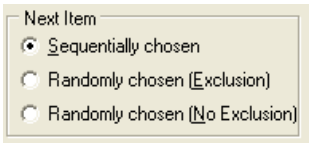
7.2.9.4 Edit the content of the list



The **Elements** section is used to select the states that will be included in the State list.

Press the (+) and (-) button for adding or deleting a state in the list. Choose the desired state in the list available from each newly created line. The only states available for selection will be those that have been previously created and defined in the States section.

7.2.9.5 Select the pattern of use of the list



Once the list has been selected as the target of a condition, the list will select the next state according to one of three criteria: Sequentially, Randomly chosen with Exclusion, or Randomly chose without Exclusion

- Sequentially: A, B, C, D, ...
- Randomly chosen (No Exclusion): D, C, C, B, A..
- Randomly chosen (Exclusion): D, C, A, B. (no repetition)

In the sequentially and randomly with exclusion cases, a state that has already been selected cannot be selected again until the list is ended/refreshed.

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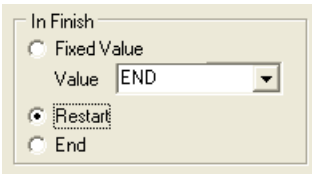
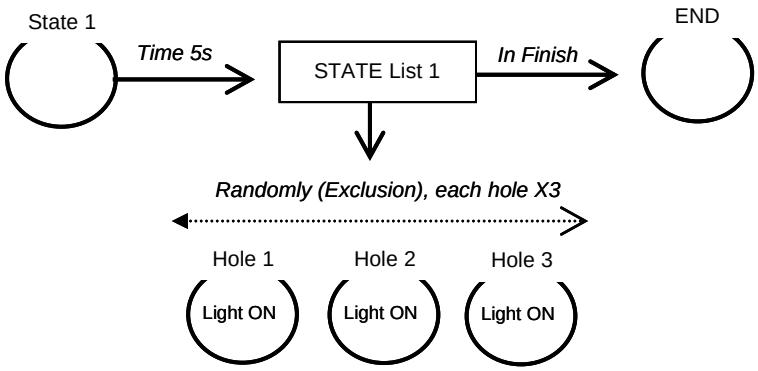


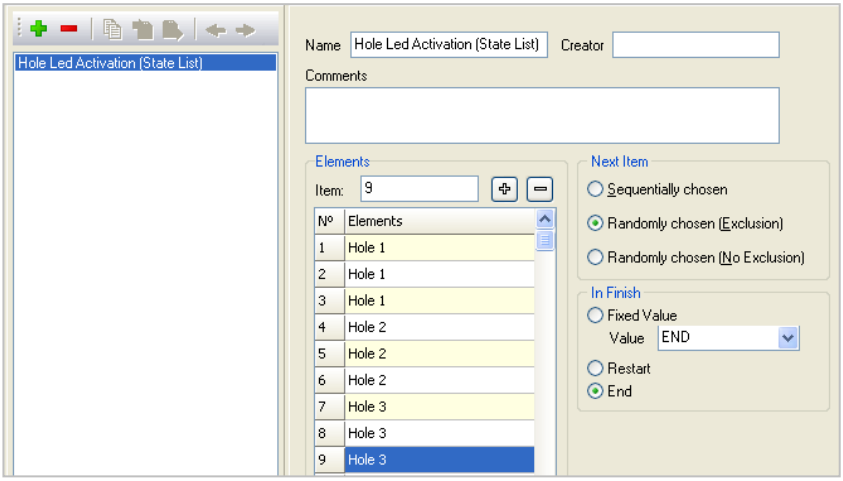
7.2.9.6 Define the finishing conditions

Once the list has ended, there are 3 possibilities:

- Fixed Value: Goes to selected state from the list of available states.
- Restart the list.
- End: Goes to END state.

In the next example, the list of state will set the occurrence of a light in one of 3 holes selected from a 5/9-hole box. In our protocol with 9 trials, the subject should receive the visual stimulus 3 times in each hole in a random order and then, go to END.





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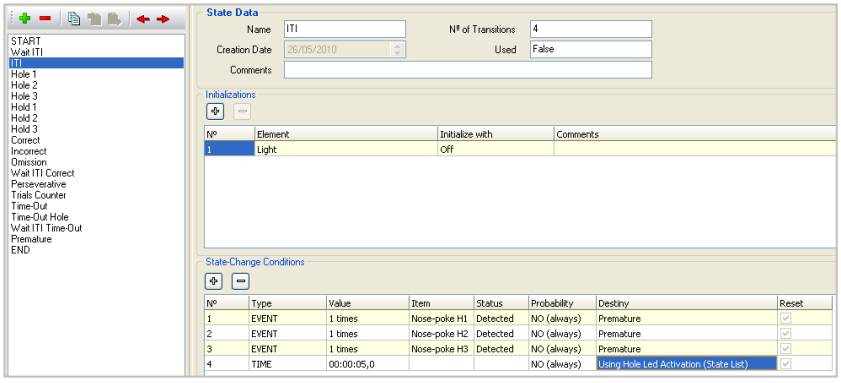
From the States section, configure in the INI state a Time state-change condition of 5s and select the name of the created State list (here "Using Hole Led Activation (State List")).

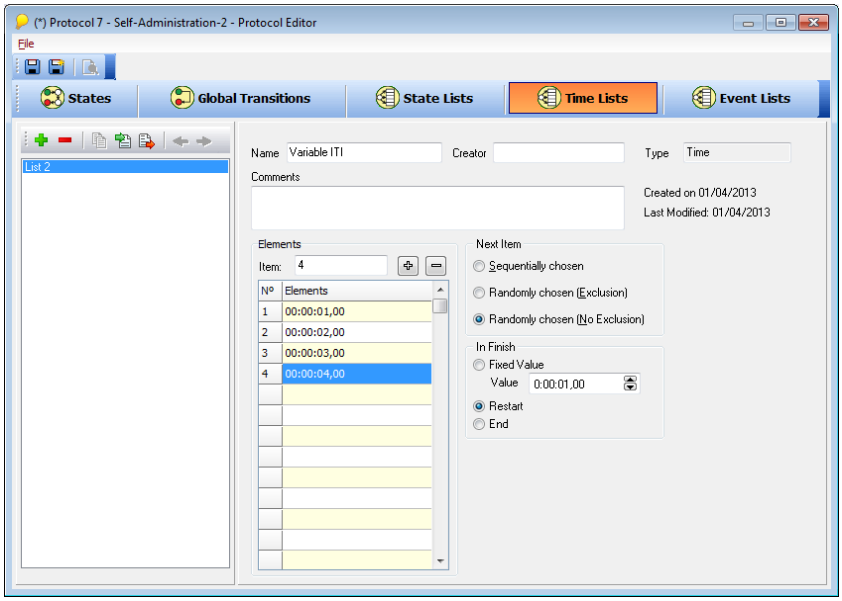


7.2.10Edit a Time or Event Lists

Time lists are used in the editing of protocols for defining a user-defined variability in one of the parameters of the State-change conditions used in the protocol.

Event lists are widely used for defining variable ratio for obtaining a reinforcement as it is the case in progressive ratio (PR) schedule for instance....

Time lists are widely used for defining variable interval time schedule or variable stimulus duration for instance...



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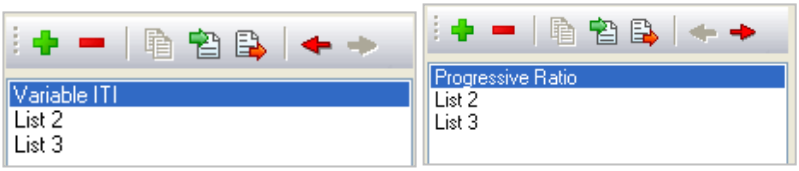
All the created Time Lists can be visualized in the list section placed on the left part of the panel.



This section is provided with a tool bar for creating, deleting, Exporting and Importing the Time lists.

7.2.10.1 Add new Time or Event List

To Add a new list, click on the **Add** button located on the top of the List section.

7.2.10.2 Delete a Time or Event List

Press the **Delete** button and accept the confirmation message for deleting the selected list.

Note: If the deleted Time or Event list was already used in one of the state-change condition defined into the States section, do not forget to change or eliminate these conditions in the corresponding states.

7.2.10.3 Export and import a Time or Event list

The Export/Import option is available to facilitate the transfer of use of the already defined Time or Event list in other protocols of the same experiment file or from other newly created experiment file.

- Select in the left panel the Time or Event list to be exported.
- Press the **Export** button. Chose a destination folder to which the exported list will be sent, and press Accept.
- The selected Time list will be exported with the *.tls extension and the Event lists with the *.els extension.
- Open the destination protocol and corresponding Time or Event section in which the user wants to transfer the exported lists.
- Press the **Import** button in the corresponding Time or Event Lists section
- Select the list to be imported and press **Accept**.

The selected protocol will be imported with the prefix "Import of…".

Important note:

Time & Event list can be exported from Locked protocols. The importation of those lists in an unlocked protocol unlocks them for allowing editing.

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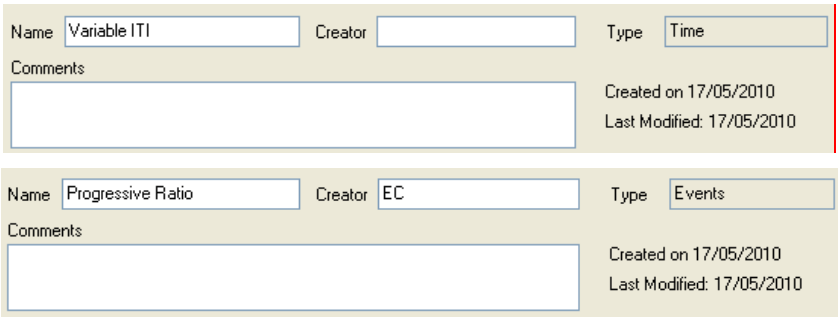
Edit the data of the Time or Event lists

The list data section allows entering user-defined name for each state, the name of the creator, and also adding comments.

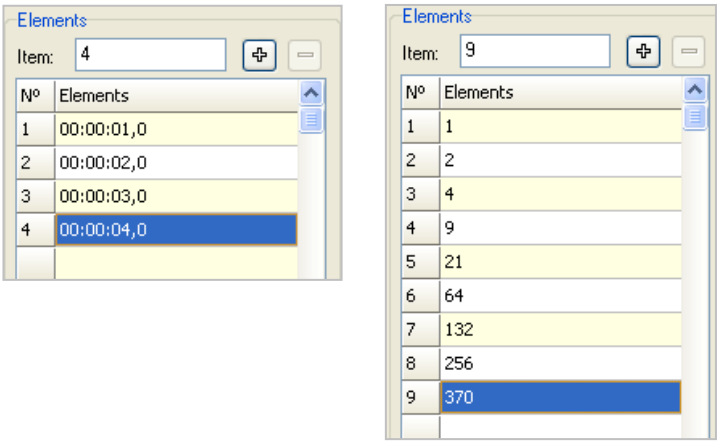


This section also shows the following not-editable items:

- **Type**: the type of the list (it can be a States, Event or Time list).
- **Creation date**: creation date of the selected list.
- **Last Modified**: date of the last modification.

7.2.10.4 Edit the List content

The **Elements** section is used to select the value/items that will be included in the lists.



- Press the (**+**) and (**-**) button for adding or deleting a Time value or Event number in the list.
- Edit the values of the items in the list.

In the Time list, first double clicking on the requested line on the grid, enter the value in the **Time Editor** and press the **OK** button. Time is expressed in hh:mm:ss.

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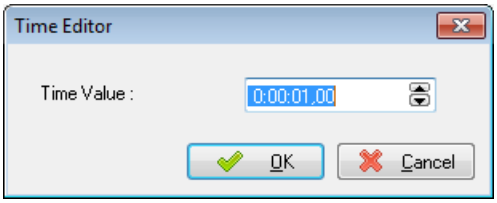
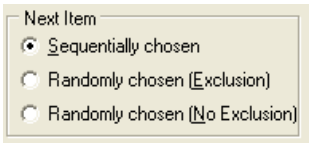


In the Event list, click on the requested line on the grid, enter a value in the line (directly by using the keyboard or indirectly by using the up and down arrows) and press the **OK** button.

Proceed in the same way for all the lines of the table.

7.2.10.5 Select the list pattern use



Once the list has been selected as the target of a condition, the list will select the Time or Event value according to one of three criteria: Sequentially, Randomly chosen with Exclusion, or Randomly chosen without Exclusion

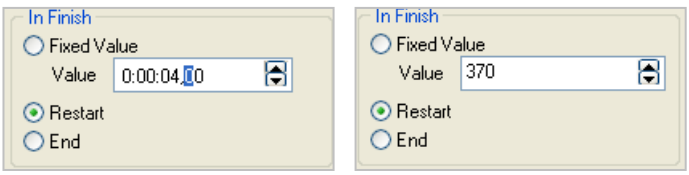
- Sequentially: item 1, item 2, item 3, ...
- Randomly chosen (No Exclusion): item 3, item 4, item 1, ... (items are picked randomly and marked so they can be selected again).
- Randomly chosen (Exclusion): item 2, item 4, item 4, item 1, ... (items are randomly selected and cannot be selected again).

In the sequentially and randomly with exclusion cases, an item that has already been selected cannot be selected again until the list is ended/refreshed.

7.2.10.6 Edit the finishing conditions of the list

Once this list has ended, there are 3 possibilities:

- **Fixed Value**: Maintain a final fixed value.
- **Restart**: start the list again.
- **End**: In OPERANT modules (CS, HO, VT), the **End** option means the end of experiment (the protocol goes to the END state). In FREEZING and STARTLE modules (CSFR and CSST), the End option means that the protocol will not stop unless a global condition ends it (if no global condition is defined, the software will crash).



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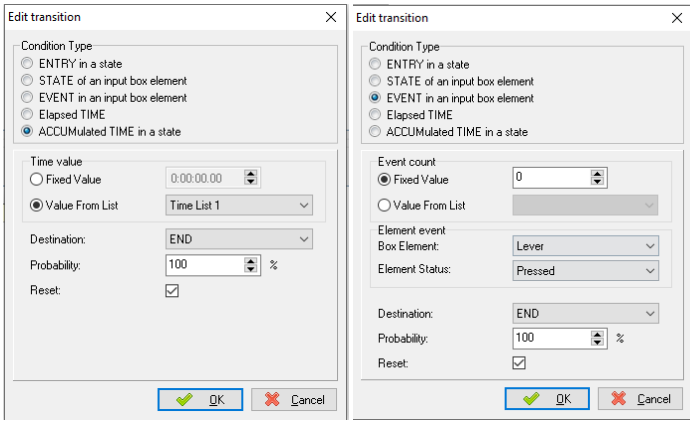
7.2.10.7 Change-state condition configuration

Once altered, the Time or Event list can be selected from the edited panel of the Elapsed time/Accumulated time and Event change-state conditions, respectively.



7.2.11 Editing the Run-Time Panels

PACKWINCS allows the execution of different protocols with different box types (self-administration, nine-hole, Vogel test, StartFear, ...) so the parameters to be shown on the screen (responses, reinforcement etc...) may vary depending on the box and protocol used.

The Run-Time panel is the set of tables and parameters that PACKWIN shows during the execution of a protocol. A configuration is set by default that can be entirely configured by the user.

From the **Protocol Information** panel, press the Edit Run-Time panels button to access to the **Run-Time Panels Editor**. The by-default Run-Time panel will be shown.

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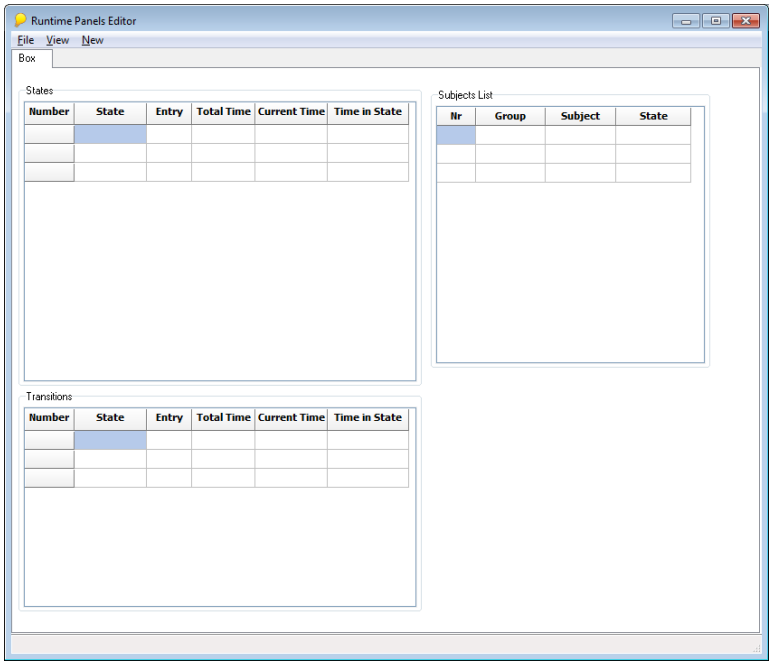

7.2.11.1 The by-default Run-Time panel

The by-default Run-Time panel configuration contains 3 Raw data tables:

- Subjects List: list of the subject that will used (sequential order).

- States: table resuming the data of the whole experiment by State.

- Transitions: table resuming the current data of the States with the transitions between States presented in chronological order of occurrence. In this table, a new line is added each time there is a transition between one State to another, providing the user with detailed information about the temporal course of the experiment.

The table provides the following data for each States of the running protocol.

Column Title	States table	Transitions table
Number	Index number of the state.	Index number of the transitions.
State	Name of the State	Name of the current State
Entry	Total number of entries into the State during the whole session	Cumulated number of entries into the current State since the beginning of the session
Total Time	Time of the last exit from the state – During acquisition, it	Last current time spent in the state

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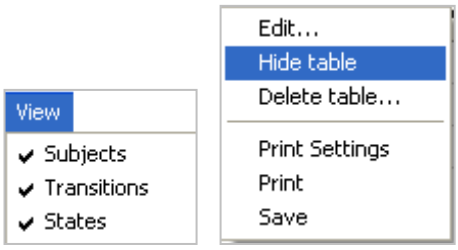
Column Title	States table	Transitions table
	also shows, in the current state, the current time elapsing from the beginning of the experiment	- During acquisition, it also shows, in the current state, the current time elapsing in the state
Current Time	Current time spent into the current State	Current time spent into the current State
Time in State	Cumulated time spend into the States from the beginning of the session	Cumulated time spend into the States from the beginning of the session

If the by-default Run-Time panel configuration does not match the specific needs of the experiment, it can be modified by selecting the elements that will be visualized in the screen as well as the parameters that will be reported in the table of results.

7.2.11.2 Edit the components of the Run-Time panel

The tables shown can be selected to be visualized or deselected to be hidden by using the **View** menu or by a right clicking on the desired element and choosing the Hide table option.



The 3 by-default Tables cannot be deleted.

It is strongly advised to keep the proposed table organization (subject table on the right and State and Transitions table on the left) for an optimal visualization of the results in the Replay report of the Single Analysis module.

7.2.11.3 Edit the tables and their content

Both Transitions and State table can be separately edited allowing an optimal flexibility for the user in choosing the parameters to be reported in each table.

Click on one of the unfilled cases of the tables (here, Transition table), click right and select **Edit.**

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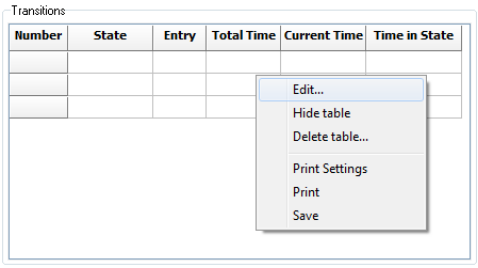
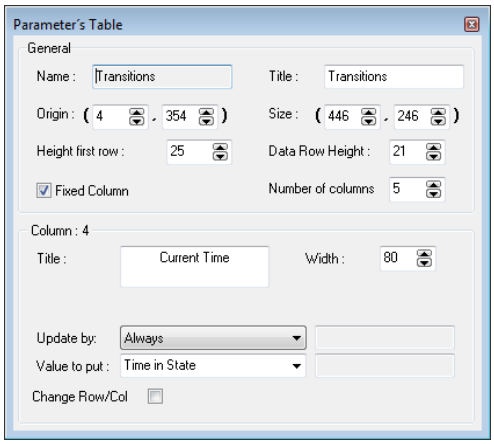
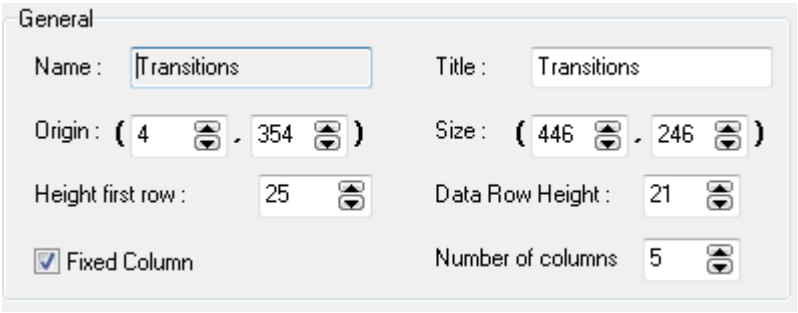


The following window will be opened.



In the **Parameter's Table** the next parameters can be configured: **General** and **Column: *n*.**

• **General** Table configuration (columns, size...)



- **Name**: name of the table
- **Title**: title of the table
- **Origin**: (x,y) location of the left/top corner of the table in the screen
- **Size**: (width, height) size of the frame
- **Height first row**: height of the first row
- **Data Row Height**: height of the data rows
- **Fixed Column**: allows fixing a column (it then takes a grey colour)
- **Number of columns**: number of columns

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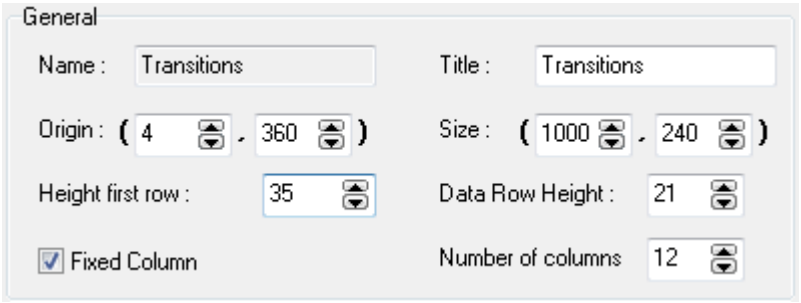
As an example in the next configuration, we add 6 new columns (number of columns), displace the point of origin to the left (Origin), expand the table in width (Size) and reduce it in height (Data Row Height).



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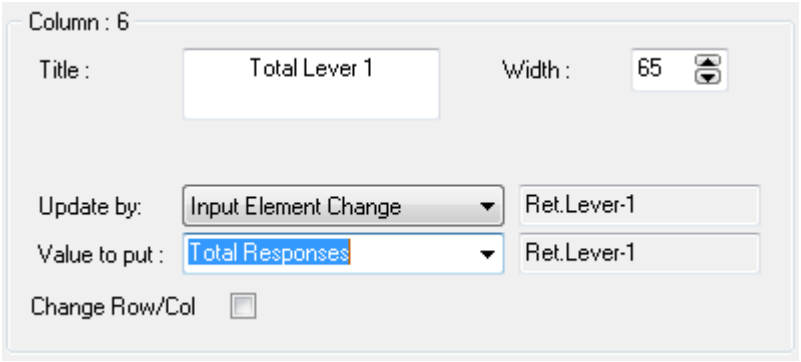
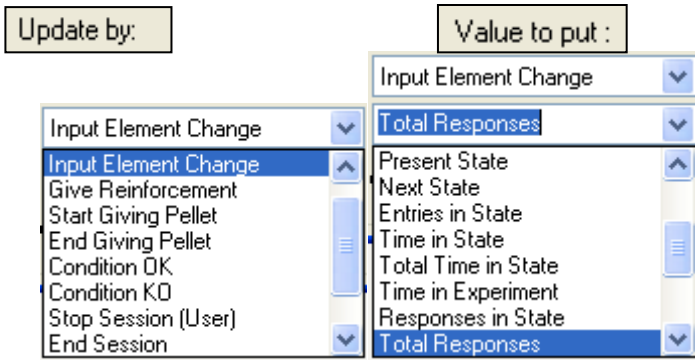
• Specific **Column** configuration (title, parameters)



- **Title**: title of the table
- **Width**: width of the column
- **Change Row/Col**: change Row/Col when the Update by condition is completed (don't have to be set by the user)
- **Update by**: condition for updating the value in the table
- **Value to put**: Values to be reported/updated

Each option chosen in **Update by** has to be associated with a parameter from the list **Value to put**



Values for **Update by** (used in both the **State** and **Transition** table):

- **Change of State**: when there is a change from one state to another
- **Input Element Change**: when the subject acts on one specific element of the box. The element has to be chosen from the **Select Element** panel.

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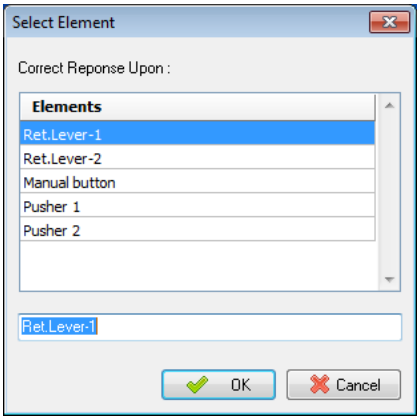

- **Give Reinforcement**: when reinforcement has been given
- **Start Giving pellet**: at the beginning of giving pellet
- **End Giving Pellet**: at the end of giving pellet
- **Condition OK**: When a State-change condition is fulfilled
- **Condition KO**: When a State-change condition is not fulfilled
- **Stop Session (User)**: When the session is stopped by the user
- **End Session**: When the session stops automatically
- **Always:** Unconditionally
- **Change Column/Row**: when a Column/Row is changed

Values for **Value to put** that can be used in the **State** table

- **Event Number**: event number
- **Present State**: name of the current state
- **Entries in State**: number of entries in the current state (accumulated from the beginning of the experiment)
- **Time in experiment**: time spent from the beginning of the experiment
- **Time in State**: current duration of each state
- **Total time in State**: accumulated duration of each state
- **Total responses in State:** total number of responses in each state
- **Total reinforcements in state:** total number of reinforcements received in each state

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- **Total time in Input Element in state:** total duration of the activation of the input element in each state accumulated from the beginning of the experiment.
- **Partial time in input element in state:** total duration of the activation of the input element in the current state.

Values for **Value to put that** can be used in the **Transition** table

- **Event Number**: event number
- **Present State**: name of the current state
- **Entries in State**: number of entries in the current state (accumulated from the beginning of the experiment)
- **Time in experiment**: time spent from the beginning of the experiment
- **Time in State**: duration of the current state
- **Total time in State**: accumulated duration of the current state
- **Given reinforcements**: number of reinforcements given in the current state
- **Total given reinforcements**: total number of reinforcements given (accumulated from the beginning of the experiment)
- **Remaining reinforcements**: number of reinforcements remaining
- **Response in State**: number of responses in the current state
- **Total responses**: total number of responses in the current state from the beginning of the experiment (accumulated from the beginning of the experiment)
- **Total time in Input Element:** total duration of the activation of the input element in the current state (accumulated from the beginning of the experiment)
- **Partial time in input element:** duration of the activation of the input element in the current state.

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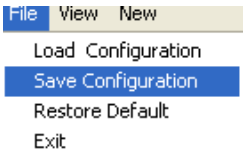
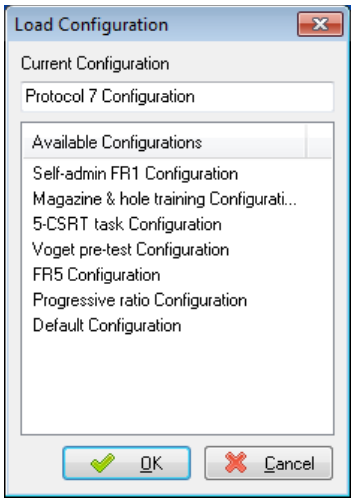


7.2.11.4 Save and Load a Run-Time panel configuration

Save the new data configuration by selecting **Save Configuration** option in the **File** menu.

Exit from the configuration module by selecting **Exit** option in the **File** menu.

The entire Run-Time panel configuration associated to the Protocols contained in the experiment file is available from the Load Configuration panel (**File/Load configuration** menu).



Each time a new protocol is created the configuration of its Run-Time panel is added to the list.

A newly created protocol can then be associated with a Run-Time Panel configuration which is shared with an already existing protocol (this saves the user from configuring it for each new protocol created).

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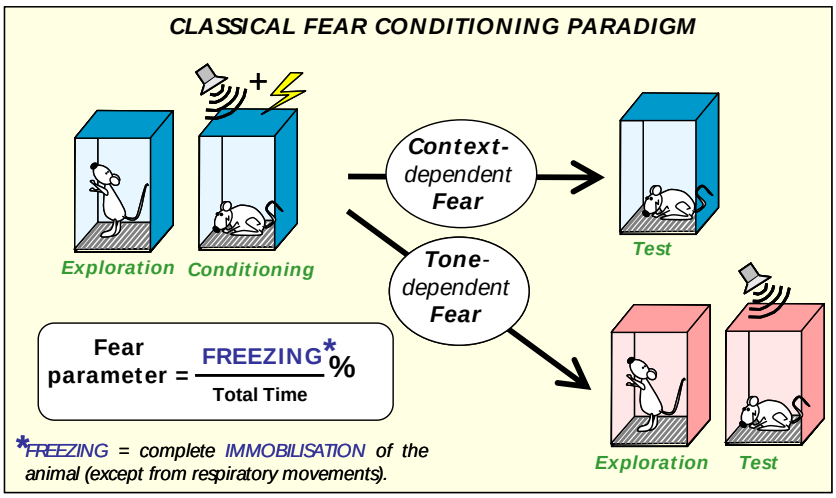
7.2.12 Example of Fear Conditioning protocol

7.2.12.1 Scientific background

Pavlovian fear conditioning is commonly used as a model for studying emotional learning and memory in laboratory animals (rats/mice). During fear conditioning, animals learn that an innocuous, non-aversive stimulus (conditional stimulus) such as a tone, predicts the occurrence of an aversive stimulus (unconditional stimulus), such as a footshock. Conditional fear is expressed in a variety of behavioral responses. In conditioned subjects, subsequent presentation of the conditional stimulus alone, according to its predictive value for the unconditional stimulus, is enough to reduce response rates. Increases in defensive responses, accompanied by autonomic and endocrine arousal, are most associated with Fear Conditioning. The most common behavioral parameter evaluated in animal experiments using the fear conditioning paradigm is freezing, defined as the complete immobilization of the animal except for respiratory movements.

Two commonly used variants of this procedure are delay and trace conditioning. In delay fear conditioning, a conditioned stimulus (CS), such as a tone is immediately followed or terminates with an unconditioned stimulus (US), such as a foot shock. In trace conditioning, a time gap is introduced between the end of the CS and the start of the US. These learning paradigms do not involve the same brain circuitries, since, for example, trace conditioning is established through hippocampal-dependent processes whereas delay conditioning does not. Moreover, a wide variety of protocols has been reported in the literature depending on animal species, strain, laboratory, aim of the study, etc.



Here is a schematical representation of protocols for delay and trace conditioning that can be found in literature. They are differentiable by the configuration of the conditioning phase, in which a time interval is present (trace conditioning) or no

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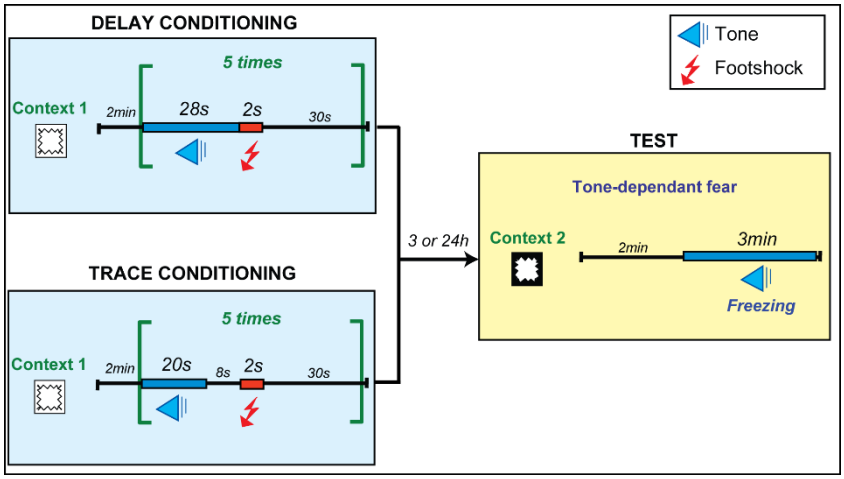
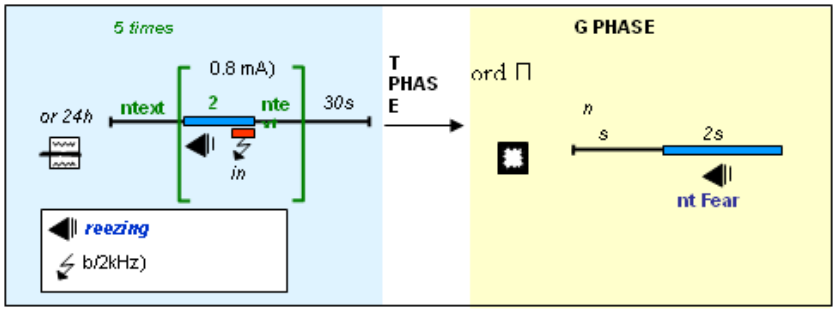
(delay conditioning) between the sound (CS) and the shock (US). The testing phase is similar for both paradigms.



The protocols can vary depending on the objectives of the study, the animal species, the animal strain, and experimental conditions. Please refer to scientific literature for choosing the protocol to apply to your experiments.

7.2.12.2 example of fear conditioning structure

Here is an example of protocols classically used for a fear conditioning study (Training and Test phases):



The procedure consists in a Training Phase followed by a Test Phase 3 or 24h after:

- In the Training phase, after starting experiment, an exploration period of 2 min is given for habituation. The exploratory period is followed by a period in which a sound is given during 30 s. The two last seconds, the animal receives an electrical shock during 2 s. After 30 s recuperation, animals are returned to its home cage.
- In the Test Phase (3 or 24 h after the Training Phase), the animal is placed in the experimental box with a new context configuration (wall with a different colour and floor with a different texture). After 2 min of habituation, the sound is given during 3 min.
- Freezing is evaluated in both phases of the experiment.

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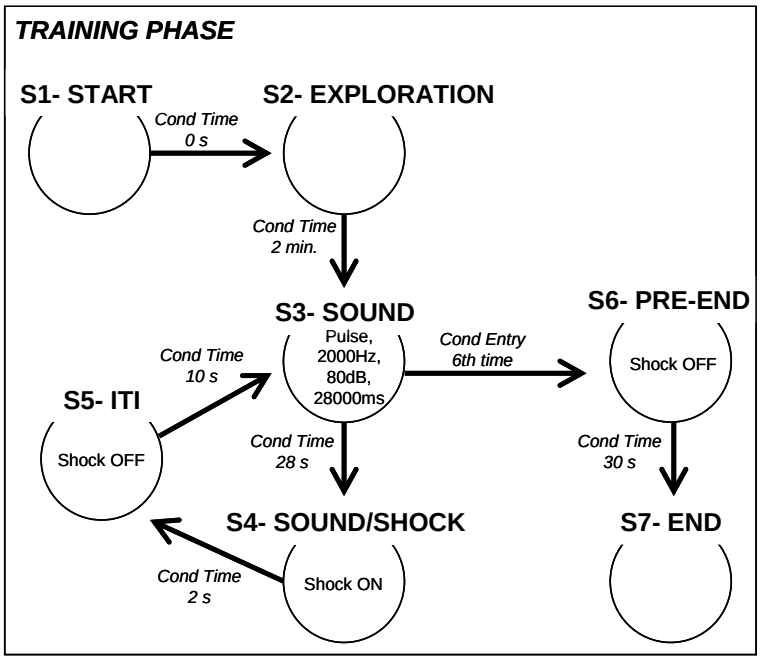
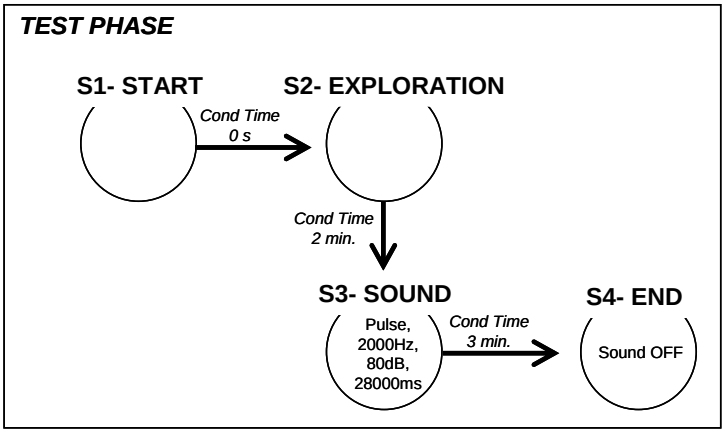
The protocols have to be previously schematized as a sequence of different states (Chain of States) with two different types of information: (i) what will happen in each state and (ii) the conditions allowing the transition between states.

Taking into account this information the Chain of State for the Training phase will be:



And the Chain of State for the Test phase will be:



See an example of how the Chain-of-state was configured with the STATE EDITOR tool in the Freezing example file provided with PACKWIN software (chapter 2.7.2).

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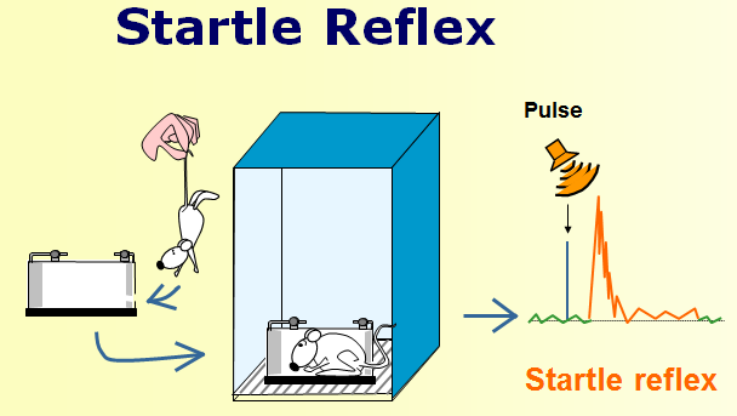
7.2.13 Example of Startle protocols

7.2.13.1 Scientific background

The PACKWIN-ST module can be used for any experiment involving the evaluation of the animal startle reflex. The startle response is a brainstem reflex elicited by an unexpected acoustic or tactile stimulus.



The most used protocols involving the evaluation of the animal startle reflex are the following:

- Prepulse-inhibition of startle reflex (PPI)
- Basal response to acoustic (or tactile) stimuli (including the hearing test)
- Fear potentiated startle reflex

7.2.13.2 Prepulse-inhibition of startle reflex (PPI)

Prepulse-inhibition (PPI) of startle reflex is commonly used as a model for studying the integrity of sensorimotor gating and attention in rodents.

PPI is the reduction of the startle response to a startling stimulus (pulse) when it is preceded by 30-300 ms by a weaker stimulus (prepulse) This response it is associated with inhibitory sensorimotor gating, a mechanism that serves to protect the earliest stage of information processing. In other words, the brain processes the prepulse signal and in turn does not process the startle stimulus. This results in reduction of the startle response. Attention and stimulus processing are fundamental components of many complexes as well as simple behaviours. Deficits in attention or the ability to detect relevant stimuli and filter out irrelevant information is central to a number of cognitive disorders such as schizophrenia, attention deficit-hyperactivity disorders, and dementia.

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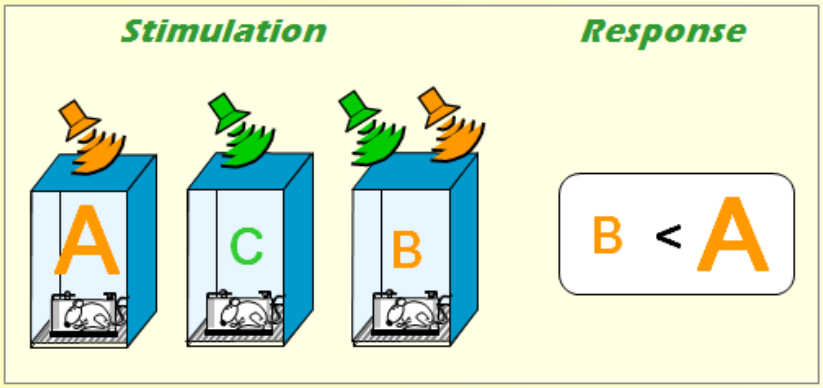
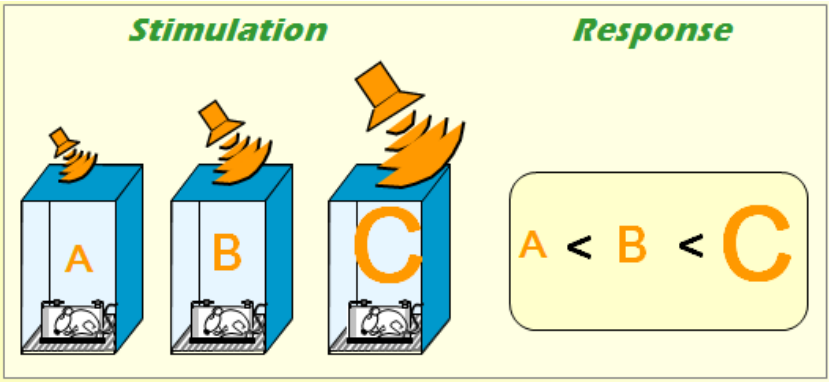


7.2.13.3 Basal response to acoustic (or tactile) stimuli

The evaluation of startle reflex response (and its habituation) to acoustic or tactile stimulus of different intensities is widely used for the detection of sensorimotor gating and hearing deficiencies in phenotyping evaluations.

Related human disease/applications: neurological phenotyping, Hyperekplexia, auditory deficits), Parkinson disease, Huntington disease, schizophrenia etc.).



7.2.13.4 Fear-potentiated startle reflex

The fear-potentiated startle reflex test is a paradigm in which the amplitude of a simple reflex is increased when presented with a cue that has been previously paired with an aversive stimulus. In the training phase, subjects are exposed to several light-footshocks pairings in a Startle box. Later, in the test phase, acoustic startling stimuli are presented consecutively to the light cue. If the association between the light cue and footshocks has been correctly learned in the training phase, light cue prior exposure increases the startle response. Inversely, in subjects with alteration of learning and memory abilities, prior presentation of the light cue does not change the startle response.

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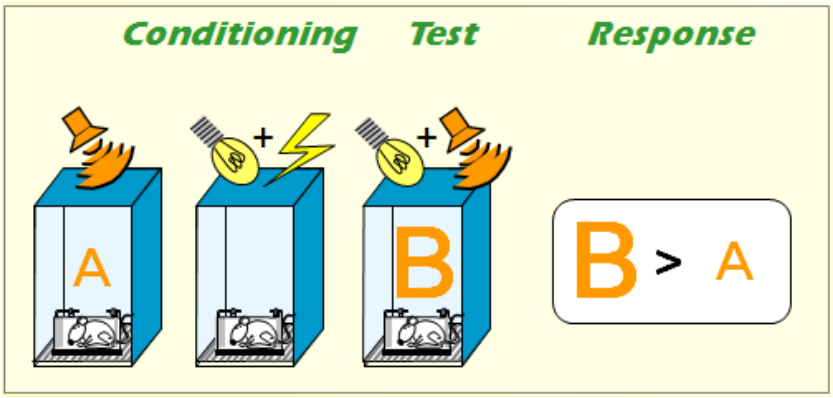
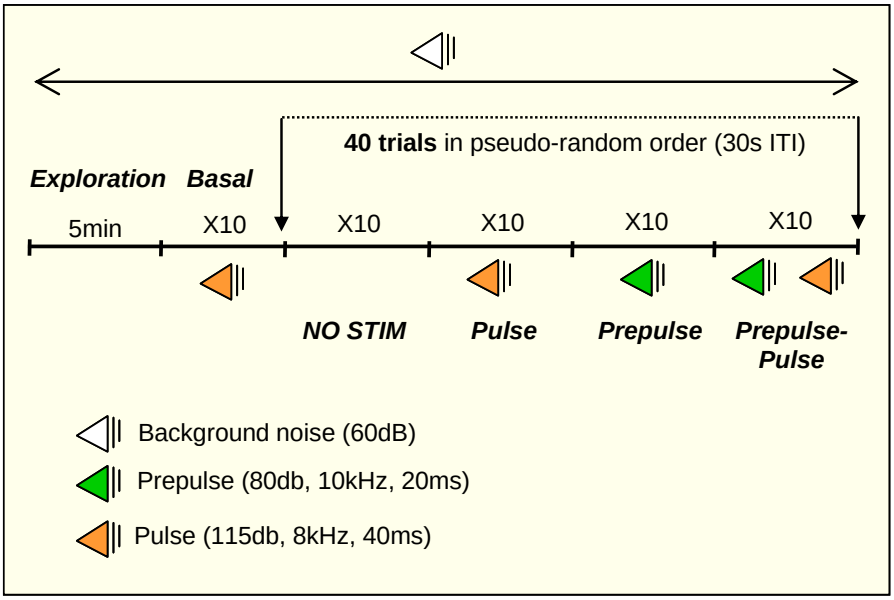


Related human disease/applications may be drug screening for memory effects, Alzheimer disease research and neurodegenerative diseases related to aging among others.

7.2.13.5 Example of startle reflex (PPI) protocol

Here is an example of protocol used for a *prepulse* inhibition startle reflex study:



- After starting experiment (START), the background noise (60 dB) is switched on and an exploratory period of 5 min is given (EXPLORATION).
- The exploratory period is followed by a period in which a basal acoustic startle reflex is evaluated in response to 10 presentations of the pulse (120 dB, 8 kHz, 40 ms) (BASAL) with an intertribal (ITI) of 30 s.
- The subjects receive then 10 presentations of 4 different trials (i) NO STIM, when no stimulus is given, (ii) PULSE, when the pulse is presented, (iii) PREPULSE, when the prepulse (80 dB, 10 kHz, 20 ms) is presented and (iv) PREPULSE-PULSE, when the prepulse is given prior to the pulse. The trials are presented in pseudorandom order with an ITI

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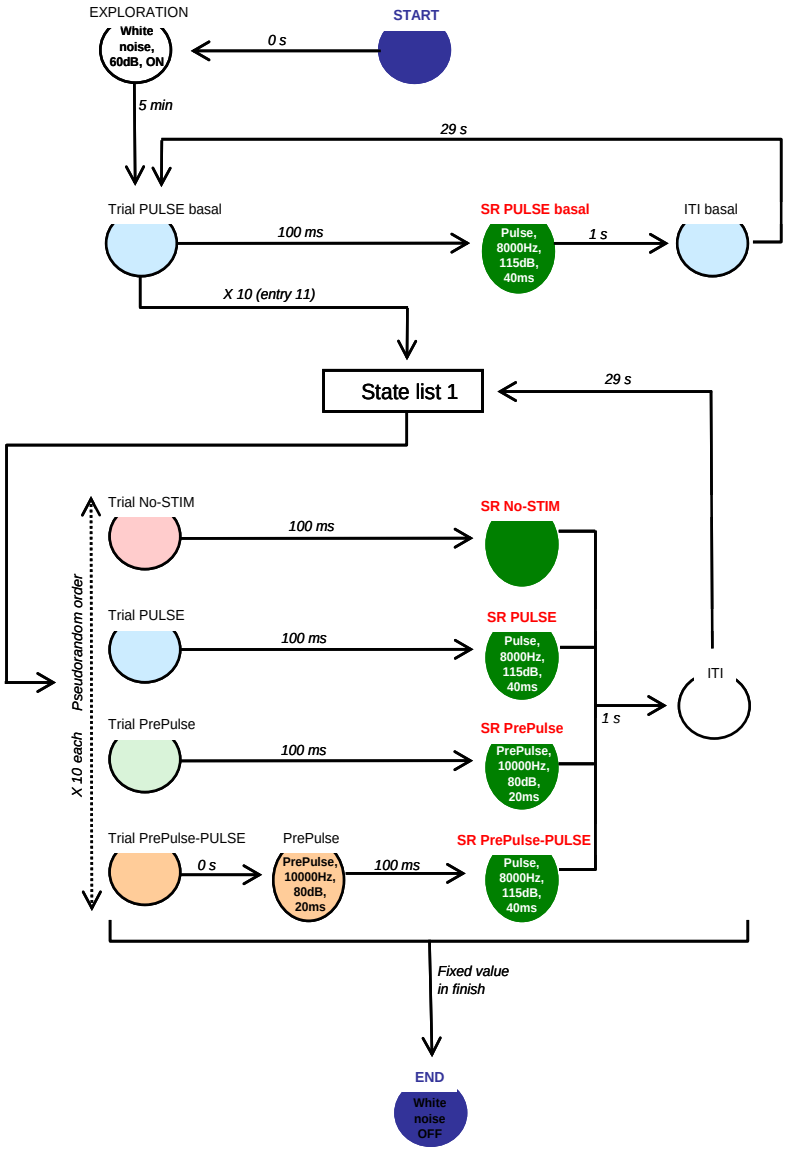
of 30 s and an interstimulus interval (between prepulse and pulse) of 100 ms.

- The startle reflex is considered to be the peak startle observed within 1 s after the beginning of the pulse presentation. The duration period of the startle signal evaluation can be chosen by the experimenter (minimal value: 10 ms).

For a better performance of the execution of the protocol, a background noise, even if at minimum volume, should always be included in all the states of a Startle protocol.

Taking into account this information the Chain of State for the Training phase will be:



See an example of how the Chain-of-state was configured with the STATE EDITOR tool in the Startle example file provided with PACKWIN software (chapter 2.7.3).

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7.3 Edit a Protocol with the PACKWINHO Assistant

This module contains two pre-set protocols in the assistant tool: the Magazine & Hole training and the 5-CSRT test assistants.

This chapter only aims to describe the specificities of the Nine Hole Module; the general functioning of the software is explained in the previous chapters.

7.3.1 Scientific background

The 5-choice serial reaction time (5CSRT) task is commonly used to evaluate attention performance using visual discrimination in laboratory animals. It represents a conditioning paradigm, involving intact attention processes. In this test, the subject has to learn to respond to a brief illumination of one of the five openings, by poking its nose inside the correct hole, in order to obtain a food reward. The time spent to get the reward and the number of errors (defined as poking the wrong hole) are inversely related to the memory the animal has of the correct hole. This means that the better the memory, the lower the time spent to poke the correct hole and the lower the number of errors. These parameters give information about the functional integrity of attentional and learning processes and are mostly altered in animal models of schizophrenia and Alzheimer diseases.

PACKWIN provides protocol templates for running standard 5CSRT task. A 5CSRT test protocol usually consists in several consecutive phases. The subject goes from one phase to the next one when a certain number of criteria are fulfilled in the previous session (number of correct responses achieved, % or correct responses etc.).

Depending on the animal species used (rat or mice) and the strain of animal used, the experimental procedure can be divided into different phases (please see corresponding literature for more details about the relevant protocol to use in your specific experimental conditions):

Below is an example of how a typical 5CSRT task may go:

A) Food deprivation

The animal is gradually deprived of 80-90% of its free feeding weight by controlled feeding over several days. This phase ensures animal motivation for performing the task.

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B) Magazine training

The nine holes of the box are occluded with metal caps and food is given in the magazine with a fixed interval of time for the animals to eat during a short period session.

C) Hole training

When the animal is reliably magazine trained, some procedures allow its training for nose-spoke. In this phase, either all the holes are illuminated, and food pellets can be placed in the holes and the magazine, or they can be automatically delivered into the magazine each time a nose-spoke occurs in one of the holes.

D) 5 choice serial reaction task training and test

This is a steady-state procedure evaluating the baseline stable attentional performance produced by daily sessions of training that gradually approximates the desired task parameter.

Briefly, during the trial, short-lasting stimuli are given in pseudo-randomized order in one of the 5 holes. The animal is required to detect brief flashes of light occurring in one of the five holes. To do it, and thus receive a reinforcement (pellet/drop), it must respond in the appropriate aperture before a certain period (limited hold) has elapsed. If the animal fails to respond, responds in the wrong hole, or at the inappropriate time, then a short period of darkness occurs (time-out) as punishment. Each trial is discrete, being separated by an intertrial interval (ITI), and there are typically 100 trials in a session.

During training, the light stimulus duration into the holes and the limited hold period are quite long (as an example: 30 s and 60 s respectively) This way the animal acquires the main task contingencies while placing little pressure on attentional capacities.

As performance reaches suitable criteria, the task parameters become gradually more stringent until the attaining the target parameters for attentional processes: commonly 0.5 s light stimulus duration and 5s limited hold period.

Improvement in performance is usually smoothly continuous until the stimulus duration is less than 1 s, which often corresponds to the duration if the animal's latency to respond correctly. However, further training over additional sessions generally enables stable, accurate performance to be attained.

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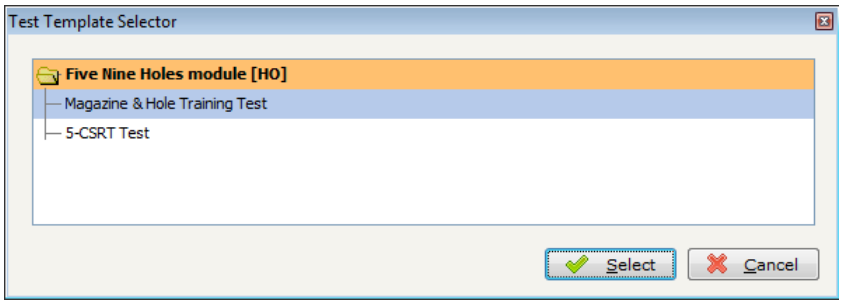
7.3.2 Nine Hole Protocol assistants

Press the **Assistant Tool** option to access to the Test Template selector.

PACKWIN provides two straightforward protocol templates for both Hole & Magazine training and 5-CSRT task (Five-Choice Serial Reaction Time) procedures.



Choose the **Test Template** to be used in the **Test Template Selector** panel by clicking on one of the options proposed and pressing the **Select** button.

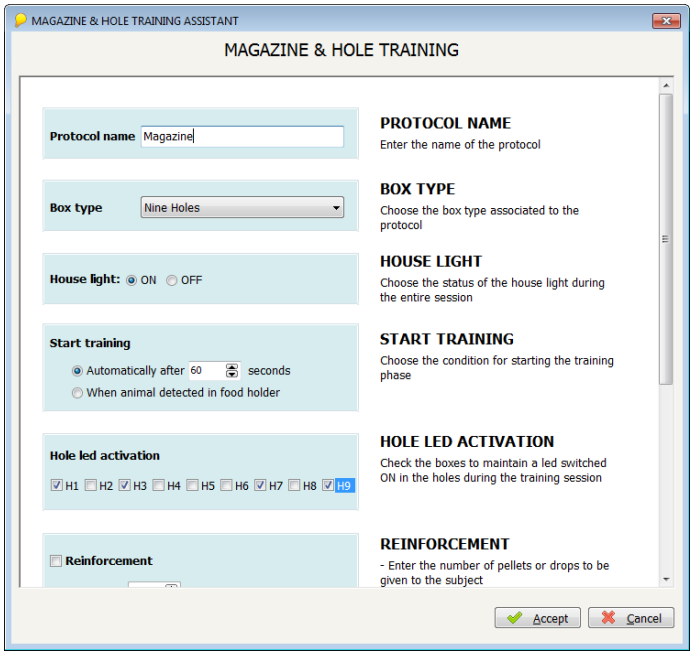
In the selected Test Template, check, uncheck, select, or fulfil the available options depending on the requirements of the requested protocol.

When both the Nine Hole and Customized modules are purchased, a protocol generated with a Nine Hole template can be opened afterwards with the State Editor Tool. This feature allows the user to have access to the underlying Chain-of-State structure of the protocol for customized modifications.

Note: A Nine Hole protocol modified with the Customized State Editor will not be allowed to be opened again with the original Nine Hole assistant template.

7.3.2.1 Magazine & Hole training Assistant

TEMPLATE



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The **Magazine & hole training** assistant provides all the tools needed for the configuration of standard training protocols which occur prior to the 5-CSRT task (Magazine training and Hole training protocols). Some instructions and explanations are displayed on the right side of the configurations box to facilitate in the editing of the protocols.

Since the panel is self-explanatory, only some critical comments about the different steps will be indicated for editing a protocol using this assistant:

PROTOCOL NAME

A protocol name is given by default, but it can be changed by the user in the **Protocol name** section

BOX TYPE

It is mandatory to select a Box type from the available options proposed in the **Box type** section. The available box types will depend on the specific box configuration purchased with the software: Nine Holes (9 holes box with 1 pellet dispenser) or Nine Holes Nine Pellets (9 holes box with 9 pellet dispensers). The Nine Holes Nine Pellets is no longer available commercially. We keep the option available in the software to ensure compatibility with existing customers using this box.

HOUSE LIGHT

The status of the house light set in the **House light** section will be applied during the whole session (exploration period included, if existing).

START TRAINING

When the **Automatically after … seconds** option is chosen in the **Start training** section, setting the value at zero implies that the training phase begins immediately after starting data acquisition. Setting a value > than zero implies starting the training protocol with an exploration period.

The **When animal detected in the food holder** option is only available for the Nine Hole box type (not for the Nine Hole Nine Pellets box type).

HOLE LED ACTIVATION

When boxes are checked in the **Hole led activation** section, the LEDs of the corresponding holes will be switched ON or switched OFF during the whole session (exploration period included, if existing).

REINFORCEMENT

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In the **Reinforcement** section the **Each … seconds** option is commonly used in standard Magazine training protocols when the **After any nose-poke** option is commonly used in standard Hole training protocols.

When the **After any nose-poke** option is checked, a reinforcement is only given when the animal makes a nose poke in one of the illuminated holes selected previously in the **Hole led activation** section. The holes not used in the session have to be blocked physically using the metallic caps provided with the experimental chambers.

The manual button refers to the manual button placed on the front panel of the Panlab Nine hole main Link box.

When the **After manual button pressing** option is combined with the **Each … seconds** distribution mode, the pellet interval time counter will be reset to zero each time the manual button is pressed by the user.

The **Each…seconds, Wait animal detection in food holder to allow next reinforcement** and **After manual button pressing** options are only available for the Nine Hole box type (not for the Nine Hole Nine Pellets box type).

END

When the user sets a condition of time for ending the session (**After … minutes**), this time includes the Exploration period duration (if existing).

CHAIN-OF-STATES

This chapter represents a schematic representation of the chain-of-state underlying the structure of protocol. The chain-of-state is structure of the protocol that would be configured if the STATE EDITOR TOOL was used instead of the Assistant tool and allow a better understanding of how the protocol is working.

A different Chain-of-States structure is associated with the Nine Holes and Nine Holes Nine Pellets box types.

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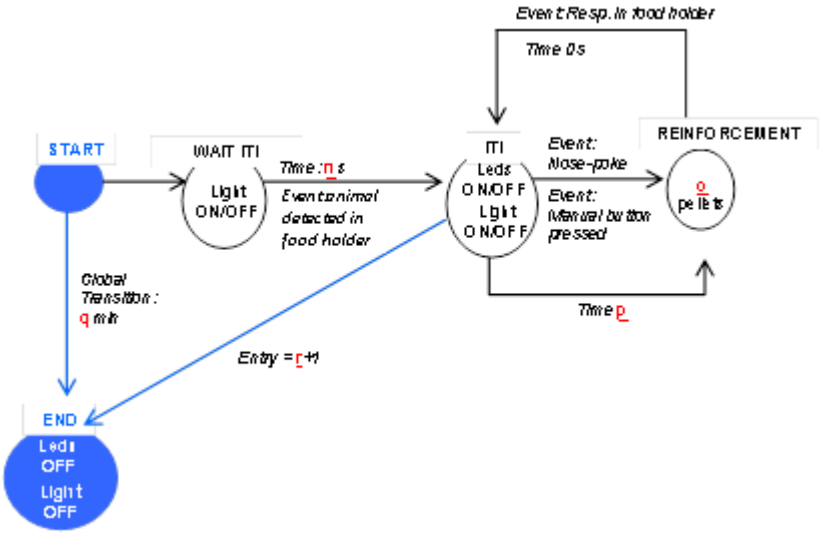
Chain-of-State for the Nine Holes box Type



n = Duration of a Habituation period defined by the **Automatically after (...) seconds** option of the **Start training** section of the assistant.

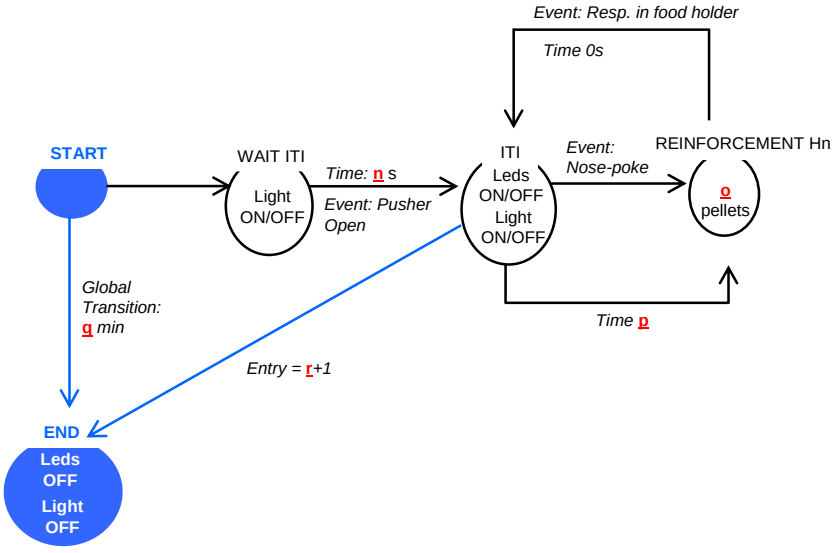
o = Number of pellets/drops given as reinforcement defined in the **Quantity** option of the **Reinforcement** section of the assistant.

p = Interval of time of pellet/drop distribution defined by the **Each (...) seconds** option of the **Reinforcement** section of the assistant.

q = Total duration of the session (including the "Habituation" phase and defined by the **After (...) minutes** option of the **END** section of the assistant.

r = Number of reinforcements defined as a condition to stop the session. Defined by the **After (...) reinforcement** option of the **END** section of the assistant.

Chain-of-State for the Nine Hole Nine Pellets box Type



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n = Duration of a Habituation period defined by the **Automatically after (…) seconds** option of the **Start training** section of the assistant

o = Number of pellets given as reinforcement defined in the **Quantity** option of the **Reinforcement** section of the assistant

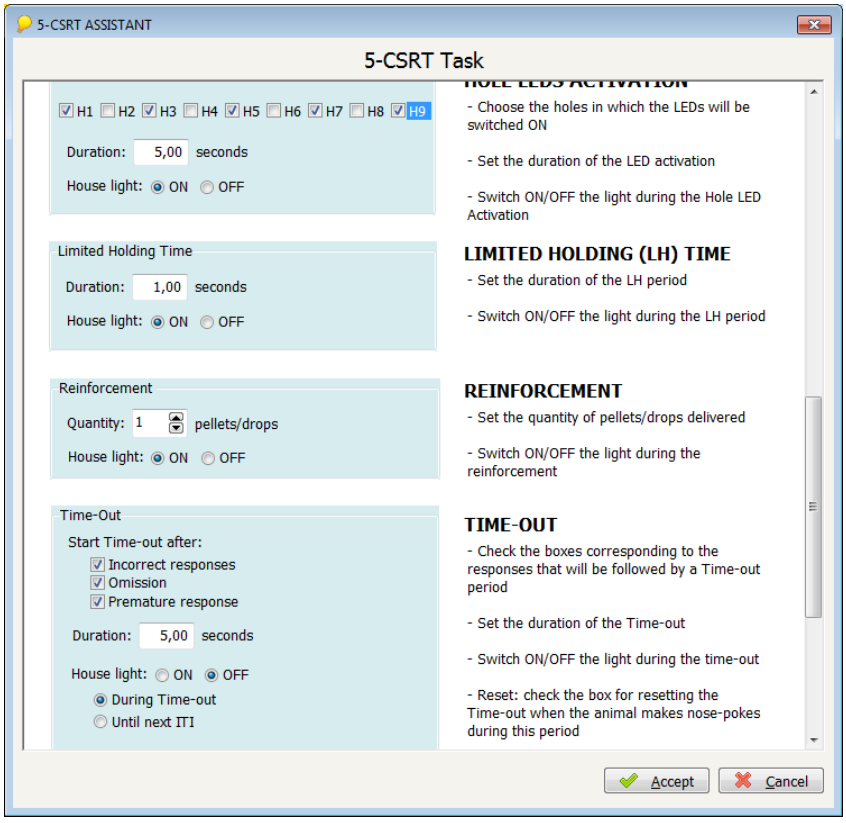
p = Interval of time of pellet distribution defined by the **Each (…) seconds** option of the **Reinforcement** section of the assistant

q = Total duration of the session (including the "Habituation" phase and defined by the **After (…) minutes** option of the **END** section of the assistant

r = Number of reinforcements defined as a condition to stop the session. Defined by the **After (…) reinforcement** option of the **END** section of the assistant

7.3.2.2 5-CSRT Test Assistant

TEMPLATE



The **5-CSRT Task** assistant provides all the tools needed for the configuration of standard protocols for 5-choice serial reaction task (5-CSRT) test.

Some instructions and explanations are displayed on the right side of the configurations box to facilitate the editing process. As the panel is self-explanatory, only some critical comments about the different steps will be indicated for editing a protocol using this assistant:

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PROTOCOL NAME

A protocol name is given by default, but it can be changed by the user in the **Protocol name** section.

BOX TYPE

It is mandatory to select a Box type from the available options proposed in the **Box type** section. The available box types will depend on the specific box configuration purchased with the software: Nine Holes (9 holes box with 1 pellet dispenser) or Nine Holes Nine Pellets (9 holes box with 9 pellet dispensers).

EXPLORATION

Setting the exploration **Duration** value at zero implies that there is no Exploration period before beginning the test.

PRIMING

When the Priming option is checked the **Quantity** of pellets cannot be set at zero.

The **Priming** option is only available for the Nine Hole box type (not for the Nine Hole Nine Pellets box type).

START FIRST ITI

When the **Automatically after ... seconds** option is chosen in the **Start first ITI** section, setting the value at zero implies that the Test phase begins with no delay after the Exploration or Priming period.

The **When animal detected in the food holder** option is only available for the Nine Hole box type (not for the Nine Hole Nine Pellets box type).

INTER-TRIAL INTERVAL (ITI)

The value of the inter-trial can be a **Fixed** value or a **Variable** value.

Any response performed into the available holes will be considered as a **Premature** response and may lead to a Time-out period or not depending on the options defined in the **Time-out** section.

HOLE LEDS ACTIVATION

When boxes are checked in the **Hole LED activation** section, the LED of one of the checked holes will be switched ON in randomly manner for each trial. The

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system will ensure that the LEDs will be switched ON the same number of times in the whole session.

During the hole illumination, any nose poke performed into the illuminated hole will be considered as a **Correct** response; any nose poke performed into the non-illuminated holes will be considered as an **Incorrect** response.

LIMITED HOLDING (LH) TIME

During the limited holding time, any nose poke performed into the previously illuminated hole will be considered as a **Correct** response; any nose poke performed into the non-illuminated holes will be considered as an **Incorrect** response; no response will be considered as an **Omission**.

REINFORCEMENT

The **Quantity** value of pellets/drops cannot be set at zero in the Reinforcement section.

TIME-OUT

A **Time-out** period can be defined after a Premature and Incorrect responses or after an Omission.

During the Time-out, the status of the house light can be changed until the end of the Time-out period (**During Time-out**) or **Until the next ITI**.

START NEXT ITI

The **When animal detected in the food holder** option is only available for the Nine Hole box type (not for the Nine Hole Nine Pellets box type).

END

When the user sets a trial condition for ending the session, all the Premature responses that has been set to be followed by a Time-out period, can be excluded from the trial counting. When the **Premature response** option of the **Time-out** section is unchecked, checking the **Premature excluded** option in the End section has no effect.

CHAIN-OF-STATE

A different Chain-of-States structure are associated with the Nine Holes and Nine Holes Nine Pellets box types.

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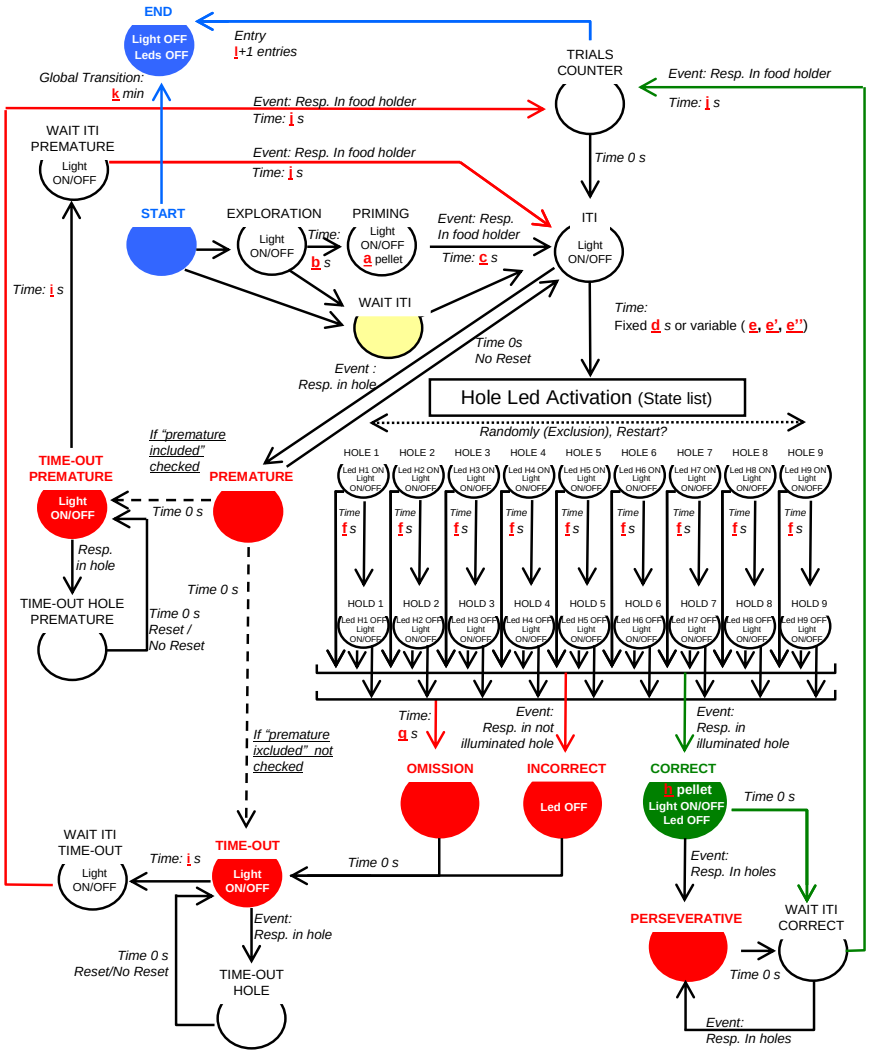
Chain-of-State for the Nine Holes box Type



a = Number of priming defined by the **Quantity** option of the **Priming** section of the assistant

b = Duration of the exploration period, defined by the **Duration** option of the **Exploration** section of the assistant

c = Time condition to begin the first TRIAL after Habituation or priming, defined by the **Automatically after (...) seconds** option of the **Start first ITI** section of the assistant

d = Fixed duration of the ITI period, defined by the **Duration** option of the **ITI** section of the assistant

e = Minimum value of the duration of the ITI period, defined by the **Min. Duration** option of the **ITI** section of the assistant when the **Variable** option is selected.

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e' = Maximum value of the duration of the ITI period, defined by the **Max. Duration** option of the **ITI** section of the assistant when the **Variable** option is selected.

e'' = Number of duration value for the ITI period, defined by the **Nb. of values** option of the **ITI** section of the assistant when the **Variable** option is selected.

f = Duration of the Holes' LED activation, defined by the **Duration** option of the **Hole LEDs Activation** section of the assistant.

g = Duration of the Holding time, defined by the **Duration** option of the **Limited Holding Time** section of the assistant.

h = Number of reinforcements given when a correct response is made, defined by the **Quantity** option of the **Reinforcement** section of the assistant.

i = Duration of the Time-out period, defined by the **Duration** option of the **Time-Out** section of the assistant.

j = Time condition to begin the next TRIAL after a response or an omission, defined by the **Automatically after (...) seconds** option of the **Start next ITI** section of the assistant.

k = Duration of the whole session, defined by the **After (...) minutes** option of the **End** section of the assistant.

l = Number of Trials defined as a condition to stop the session. Defined by the **After (...) trials** option of the **END** section of the assistant.

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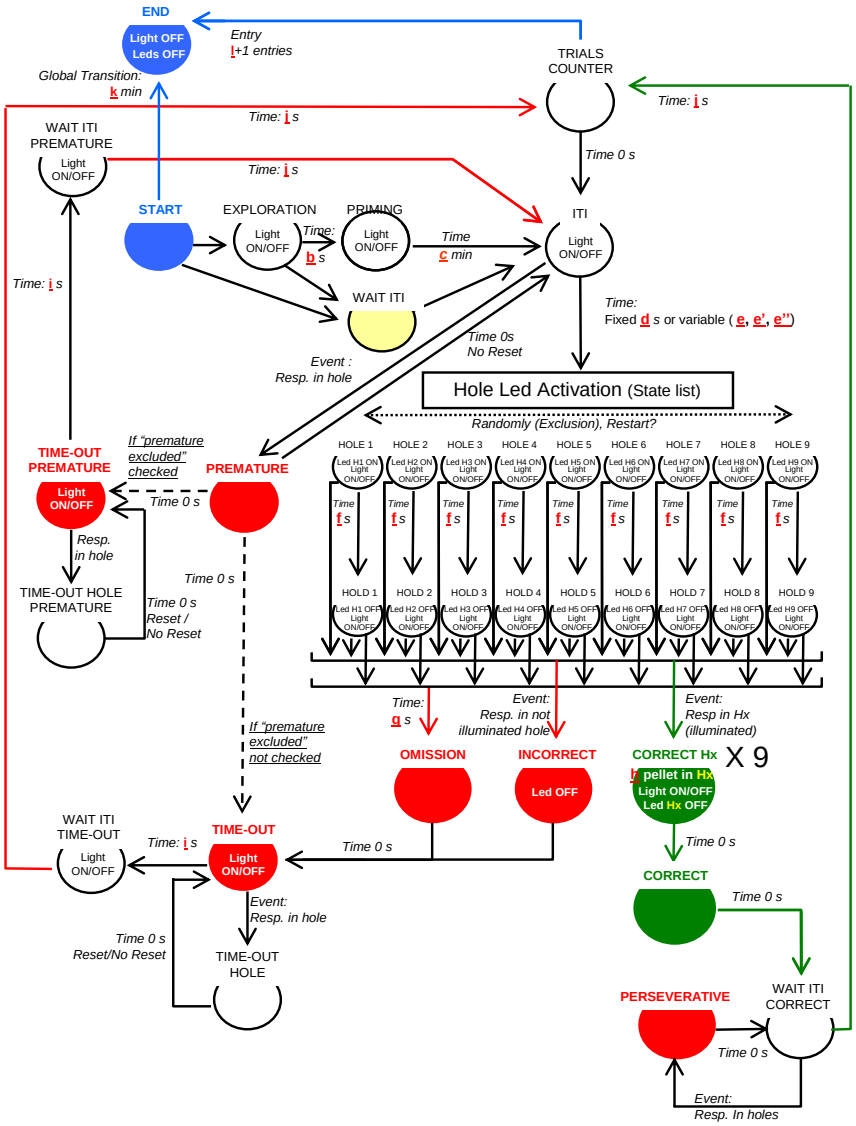
Chain-of-State for the Nine Hole Nine Pellets box Type



Same legends as the Nine hole box (see previous paragraph).

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7.4 Edit a Protocol with the PACKWINVT Assistant

The module contains two assistants: the Pre-test and the Vogel Test assistants.

This chapter only aims to describe the specificities of the Vogel test Module; the general functioning of the software is explained in the first chapters of this manual.

7.4.1 Scientific background

The Vogel test paradigm is a popular conflict model in which water-deprived rats and mice first learn to lick from a waterspout in an operant chamber. Then, usually after a period of unpunished licking, responses are punished with mild footshocks, inducing a significant reduction of drinking. In this context, administration of anxiolytics is shown to inhibit shock-induced drinking suppression. This test is classically used in rat and less commonly in mice.

PACKWIN provides protocol templates for running both Original and the Modified Vogel procedure. Variability issues in the response have been frequently reported in the literature with the original version of the test. To counteract this problem, several modifications have been described in subsequent publications. Most frequently some form of habituation period to the test chamber is used, a procedure that reduces the variability on the test day. More extreme alterations include pre-training (Pre-Test) and the formation of base-line response rates.

An example of a modified Vogel test protocol for rats can be found below and consists of four consecutive phases:

Habituation phase: on the first day of the experiment, the rats are adapted to the test chamber for a user-defined time (commonly 10 min).

Deprivation phase: after the adaptation period, the animal is exposed to a water deprivation period of 24 or 48h.

Pre-Test phase: the animal is exposed to the Pre-Test period in which it is allowed to drink in the lickometer apparatus for a user-defined time in the absence of shocks. The animals are then returned to their home-cages and allowed to drink for another short period of time. After the Pre-Test period, any animals that made fewer than a user-defined number of licks are eliminated. This is done to ensure that only animals that actually licked were included in the test. In the example protocol, the animal that does not make licks or makes fewer than 20 licks, would receive no shock during the actual Vogel Test phase, and thus there would be no conflict for the animal.

In some protocols, the Free Drink Pre-Test phase is immediately followed by the Test phase.

Vogel Test Phase: the following day, at the same hour, the Test is performed. The duration is commonly between 3-5 min. The drugs used during the test phase are

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administered before licking and shock is applied. It has previously been shown that this version of the Vogel procedure (Vogel et al. 1971) is not sensitive to variations in motivation to drink, motor effects of drugs, or analgesia.

References:

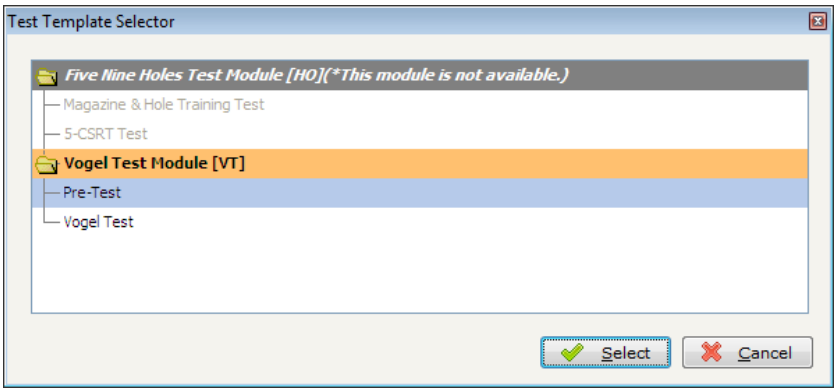
Vogel JR, Beer B, Clody DE (1971) A simple and reliable conflict procedure for testing anti-anxiety agents. Psychopharmacologia 21:1-7

Andrews JS, Broekkamp CLE (1993) Procedures to identify anxiolytic or anxiogenic agents. In: Behavioural Neuroscience – A Practical Approach – Vol II – Ed. A.Sahgal, by Oxford University Press INC., New York. Pp. 37-54.

7.4.2 Vogel Test Protocol assistants

Press the **Assistant tool** option to access to the Test Template selector.

PACKWIN provides two straightforward protocol templates for the two standard steps of the Vogel test: the Pre-Test and the Test (named here "Vogel Test").



Choose the **Test Template** to be used in the **Test Template Selector** panel by clicking on one of the options proposed and pressing the **Select** button.

In the selected Test Template, check, uncheck, select, or fill the available options depending on the requirements of the requested protocol.

When both the Vogel Test and Customized modules are purchased, a protocol generated with a Vogel Test template can be opened afterwards with the State Editor Tool. This feature allows the user to have access to the underlying Chain-of-State structure of the protocol for customized modifications.

Note: A Vogel Test protocol modified with the Customized State Editor will not be allowed to be opened again with the original Vogel Test assistant template.

7.4.2.1 Pre-Test Assistant

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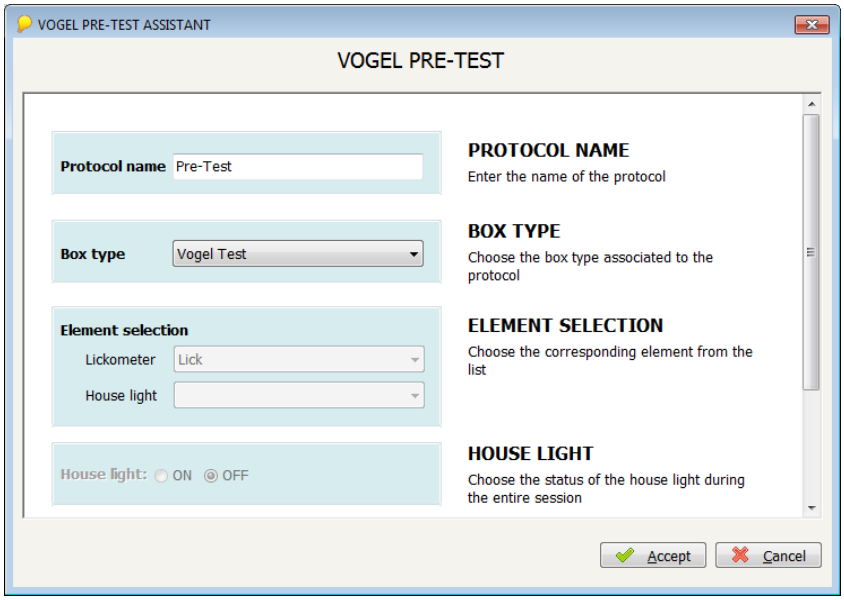
TEMPLATE



The **Vogel Pre-Test** assistant provides all the tools needed for the configuration of standard protocols of Pre-Test, which occur prior to the Test Phase of the Vogel test. Some instructions and explanations are displayed on the right side of the configurations box to facilitate the editing of the protocols.

As the panel is self-explanatory, only some critical comments about the different steps will be indicated for editing a protocol using this assistant:

PROTOCOL NAME

A protocol name is given by default, but it can be changed by the user in the **Protocol name** section.

BOX TYPE

It is mandatory to select a Box type from the available options proposed in the **Box type** section. The available box types will depend on the specific box configuration purchased with the software: Vogel Test box (with pre-defined elements: a lickometer and a shocker) or any kind of Operant boxes from the Panlab family (with user-defined elements). The options of the panel with be available (or not) depending on the Box Type selection.

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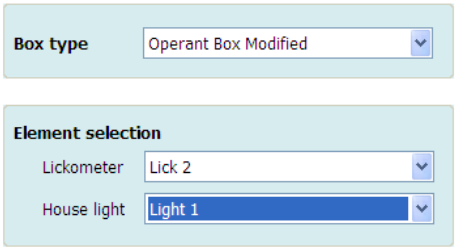
ELEMENT SELECTION

This selection allows the user to select the elements being utilized in the Pre-Test protocol.

When the Vogel Test box type us used, the Element selection section is not available for changes. The Vogel Test box is provided with a standard and unchangeable structure consisting basically in a lickometer and a shocker.

When any other Operant boxes from the Panlab family is used, the user should indicate which elements will be used as Lickometer (required) and as House light (optional). A lickometer selection is required to allow the protocol to be accepted.



HOUSE LIGHT

This option is only available when using an operant box-like system, including (at least) a house light stimulus in the list of the elements of the box. In this example, the status of the house light set in the **House light** section will be applied during the whole Pre-Test session.

DURATION

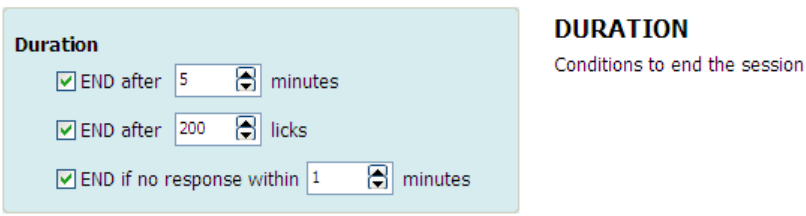
This section defines the duration of the Pre-test period by setting the following configurations:

END after a user-defined time

END after a user-defined number of licks

END if the animal makes no response within a user-defined time.

These 3 configurations are not exclusives: if several of them are checked, the first condition that is fulfilled will end the session.



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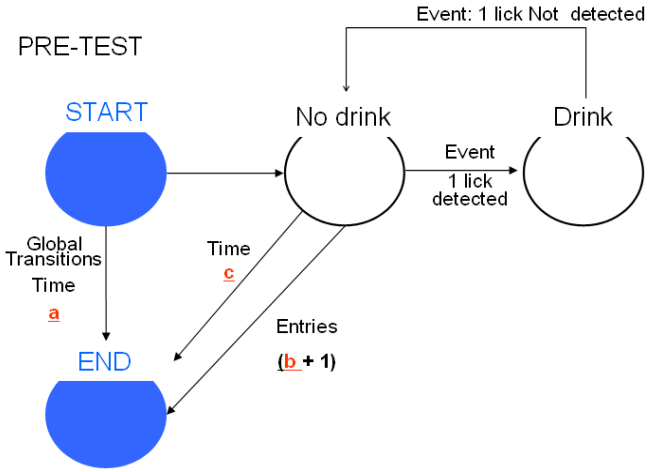
During this Pre-Test time, the animal is allowed to drink without receiving any shocks.

CHAIN-OF-STATES

This chapter represents a schematic representation of the chain-of-states underlying the structure of the protocol. The chain-of-sate structure of the protocol would be configured if the STATE EDITOR TOOL was used instead of the Assistant tool and would allow for a better understanding of how the protocol is working.

Below is the illustration of the Chain-of-States structure underlying the Pre-Test protocol.



a = time set in the **END after *x* minutes** option

b = number of licks set in the **END after x licks** option

c = time set in the **END if no response within x minutes option**

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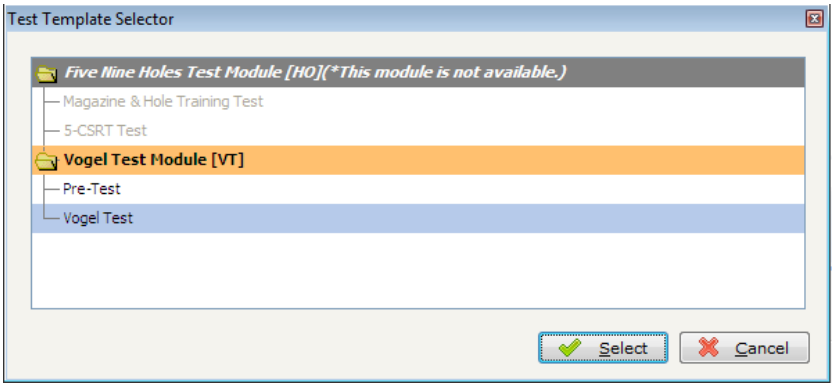
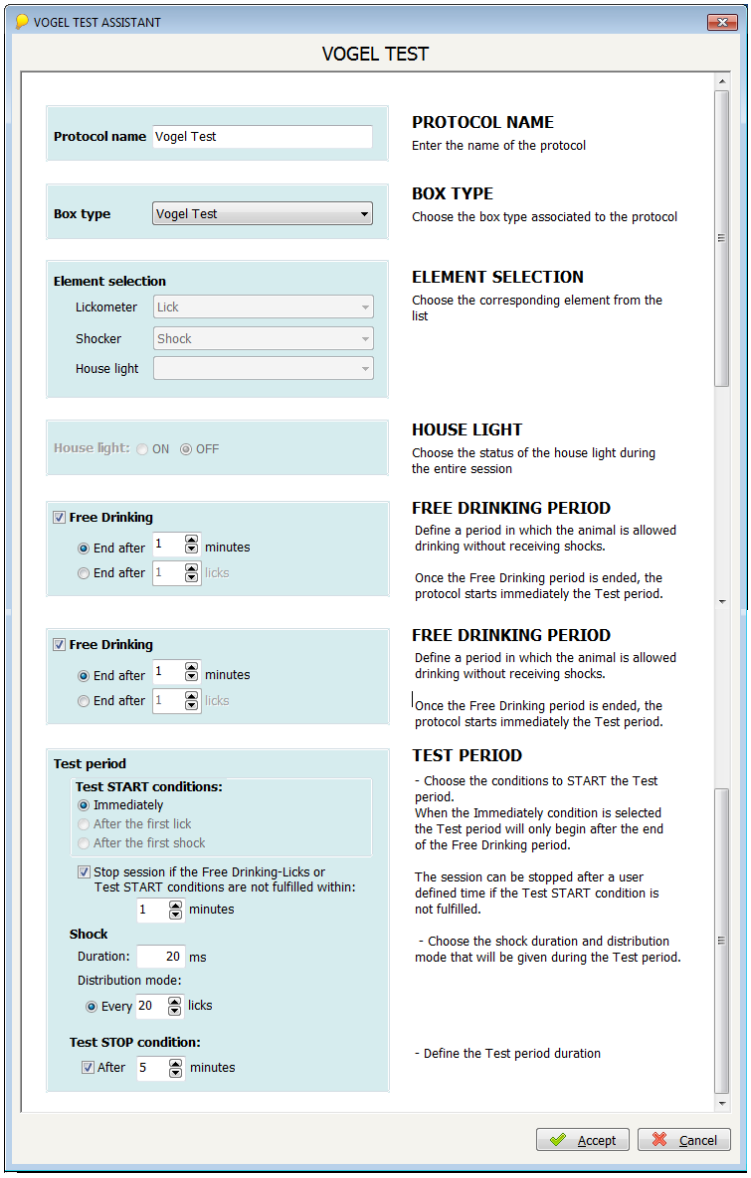
7.4.2.2 Vogel Test Assistant



TEMPLATE



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The **Vogel Test** assistant provides all the tools needed for the configuration of the Test phase of the Vogel test in which the animal receives shocks after a user-defined number of licks.

Note: additionally to the Test period, the Vogel Test template also provides the possibility to set a previous Free Drinking period in which the animal is not receiving shocks (very similar to the Pre-Test period but leading automatically to the Test period depending on some options that can be chosen by the user).

Some instructions and explanations are displayed on the right side of the configurations box to facilitate their editing. As the panel is self-explanatory, only some critical comments about the different steps will be indicated for editing a protocol using this assistant:

PROTOCOL NAME

A protocol name is given by default, but it can be changed by the user in the **Protocol name** section.

BOX TYPE

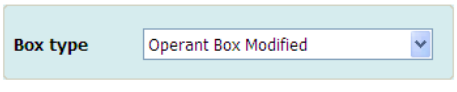
It is mandatory to select a Box type from the available options proposed in the **Box type** section. The available box types will depend on the specific box configuration purchased with the software: Vogel Test box (with pre-defined elements: a lickometer and a shocker) or any kind of Operant boxes from the Panlab family (with user-defined elements). The options of the panel will be available (or not) depending on the Box Type selection.

ELEMENT SELECTION

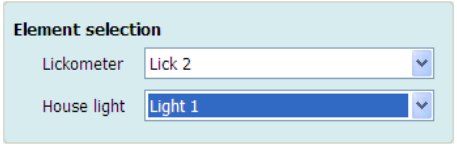
This allows the user to select the elements being utilized in the Pre-Test protocol.

When the Vogel Test box type is used, the Element selection section is not available for changes. The Vogel Test box is provided with a standard and unchangeable structure consisting basically of a lickometer and a shocker.

When any other Operant boxes from the Panlab family are used, the user should indicate to the software which elements will be used as Lickometer (required) and as House light (optional). A lickometer selection is required in order to allow the protocol to be accepted.



BOX TYPE
Choose the box type associated to the protocol



ELEMENT SELECTION
Choose the corresponding element from the list

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HOUSE LIGHT

This option is only available when using an operant box-like system, including (at least) a house light stimulus in the list of the elements of the box. In this example, the status of the house light set in the **House light** section will be applied during the whole session: Free Drinking period (if existing) + Test period.

FREE DRINKING

The Free drinking period can end after a user defined time (**End after *x* minutes** option) or after a user-defined number of licks (**End after *x* licks** option).

Note1 : When the FREE DRINK PERIOD is unchecked or the **End after *x* minutes** option chosen with a 0 minute value, the test begins with a WAIT TEST period which duration will depends on the options set in the Test PERIOD section.

Note: The value of 0 licks is not accepted for the **End after *x* licks** option: the number of licks has to be ≥ 1.

TEST PERIOD

The TEST PERIOD begins depending on the user-selected following options:

Immediately – immediately after the end of the FREE DRINKING period (if existing) or when starting the session.

After the first lick – after the first lick registered during the WAIT TEST period. Only available when the Free Drinking period is unchecked.

After the first shock – after the first shock registered during the WAIT TEST period. Only available when the Free Drinking period is unchecked.

The session can be stopped after a user defined time if the Test START condition is not fulfilled. The minimal value for this time is 1 minute. (If the user replaces the value with "0" using the keyboard, the "0" will be automatically replaced by "1" when the "Accept" button is pressed.)

During the Test period, the subject will receive a shock after a user-defined number of licks. The duration of the shock has to be ≥ 10 ms. The value of 0 licks is not accepted in the **Every *x* licks** option: the number of licks has to be ≥ 1.

The Test period duration is over when the time set in the **After *x* minutes** option of the Test STOP condition section has elapsed. The minimal value for this time is 1 minute. (If the user replaces the value with "0" using the keyboard, the "0" will be automatically replaced by "1" when the "Accept" button is pressed.

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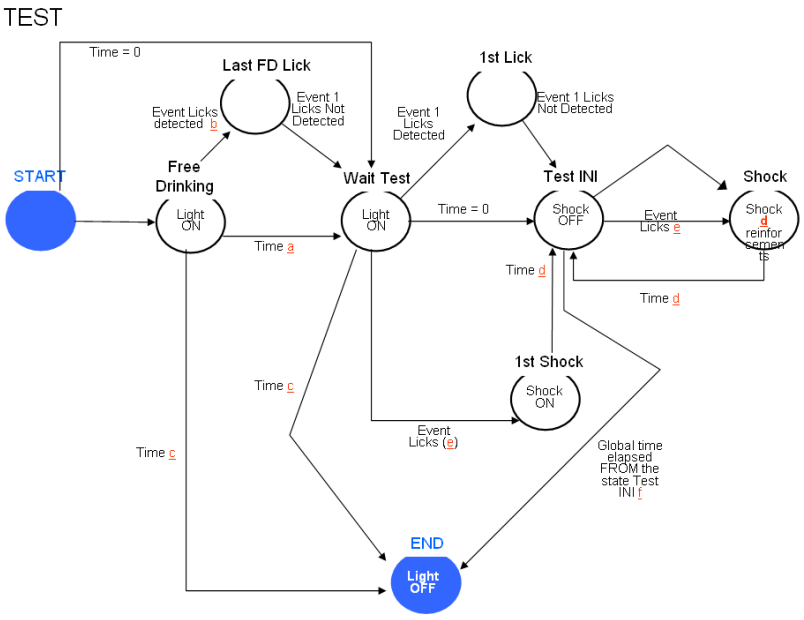
CHAIN-OF-STATE

Below is an example illustration of the Chain-of-States structure underlying the Vogel Test protocol template.



a = time set in the **END after *x* minutes** option of the FREE DRINKING period

b = number of licks set in the **END after *x* licks** option of the FREE DRINKING period

c = time set in the **Stop session if the Test START conditions is not fulfilled within x minutes** option of the TEST PERIOD

d = time set in the **Duration: x ms option** of the shock in the TEST PERIOD

e = time set in the **Every x licks** of the shock distribution mode in the TEST PERIOD

f = time set in the **Test STOP condition** of the TEST PERIOD

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8 Data Acquisition

Once protocols are designed and the subjects and groups are defined, the experiment is ready to start data acquisition sessions. The data acquisition panels and process may differ between OPERANT and STARTLE/FREEZING modules. Common steps and differences will be described in the current chapter.

8.1 Conditions For Acquisition Button Availability

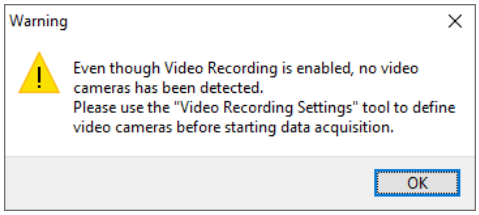
The Acquisition button is only available when the following conditions are fulfilled:

- The TRIAL version of the software is used before it expires.
- The USB license key is connected to the computer when the trial time has been elapsed.
- The Boxes definition/connection has to be checked at least one time each time the PACKWIN software is opened.
- At least one subject is available in the Subjects data base.
- At least one correct protocol is available in the Protocols organizer. The protocols are considered correct when the 2 following conditions are fulfilled:
    - The protocol has been related to a specific box definition.
    - The START state of the protocol leads to another state.

8.1.1 Synchronized Video Recording

In order to use the Synchronized Video recording feature the following conditions must be fulfilled in addition to those above:

- The Record-it! Media application must be running.
- A Video camera configuration file must be loaded in the Record-it! Media application.
- PACKWIN must be connected to the Record-it! Media application.
- PACKWIN must know the list of cameras to be used during the data acquisition.



Refer to the chapter 12.2 in order to fulfil these conditions before running data acquisition.

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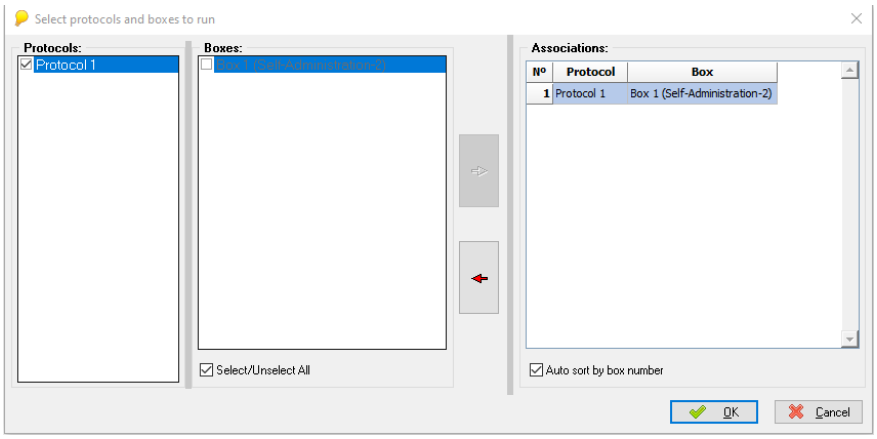
8.2 Associate Protocols and Boxes

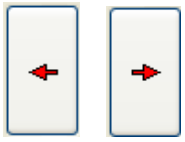
When the Acquisition button is pressed, the user has to indicate in the **Select protocols and boxes to run panel** which protocol will be entered into the experimental boxes.



The **Select protocols and boxes to run** panel allows assigning a protocol to the experimental boxes configured during the **Box Definition** process.

The panel is composed of three grids:

- **Protocols** grid (left): shows the defined protocols contained in the experiment file.
- **Boxes** grid (central): shows the available experimental boxes.
- **Associations** grid (right): shows the associated protocols and boxes.



Select a Protocol and a Box, and then press the right red arrow for associating them.

Use the left red arrow for disassociating the protocols and Box listed in the Associations grid.

Please note that only OPERANT modules allow independent selection of protocols for each boxes, meaning that a different protocol can be executed in each box. The FREEZING and STARTLE modules are designed to execute the same protocol in all boxes so a different protocol cannot be selected for each box. If the user would like to select a new protocol for the boxes, the association need to be undone first.

Press ***Ok*** button when done.

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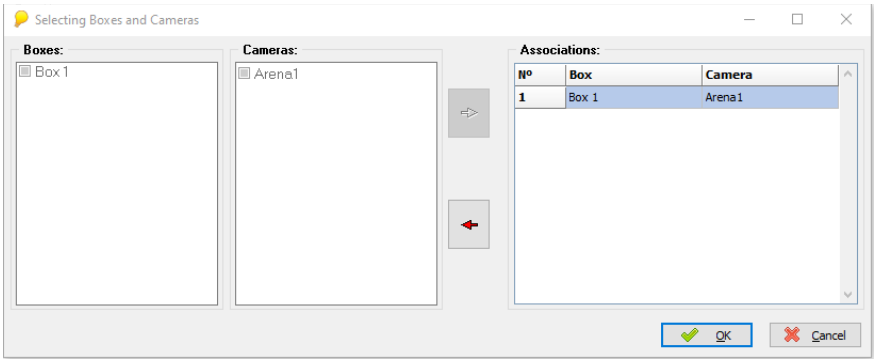
8.3 Pairing Boxes and Cameras

When the Acquisition button is pressed in the **Associate Protocols And Boxes** window, the **Selecting Boxes and Cameras** panel will appear. Within this window the user must indicate which camera is paired to each experimental chamber.



The panel is composed of three grids:

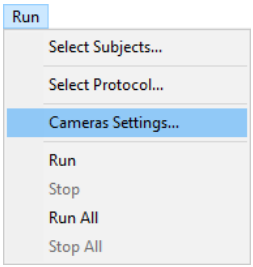
- **Boxes** grid (left): shows the enabled boxes for the current data acquisition session.
- **Cameras** grid (central): shows the available video cameras defined in the camera config file loaded into Record-it! Media application, by the **Video Recording Settings** tool (see chapter 12.2 for more information).
- **Associations** grid (right): shows the associated cameras and boxes.

Once a Camera and a Box are selected, a pairing will be added automatically.

Use the left red arrow to disassociate a Camera/Box pairing listed in the Assignations grid.

Press ***Ok*** button when done.

It is very easy to identify which boxes have an associated camera and which do not, thanks to the icon that is paired with each tab. In the example image below, box number 3 has not yet been associated with a camera.



The pairing of boxes and cameras can be done at any time. Select the "Cameras Settings..." menu option in the "Run" main menu to open the **Assign Boxes and Cameras** panel.

Things to keep in mind during data acquisition:

a) if a box is running a protocol, it cannot be paired with a camera.

b) if a camera is already recording a video file, the pairing cannot be undone.

c) The conditions above also applies to boxes linked by the yoked procedure.

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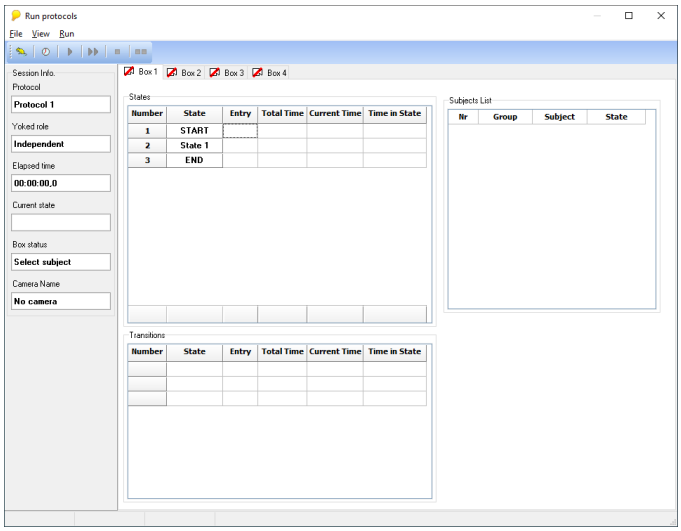
8.4 Run-Time Panel & Data Acquisition Process

The Acquisition Run-Time panel and data acquisition process differ among the different PACKWIN modules used.

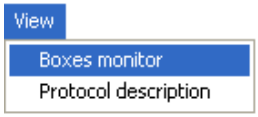
8.4.1 PACKWIN Operant Modules (CS, HO and VT)

The **Run protocols** window of an Operant Conditioning System is composed of a main menu, a tool bar, a Session Info. Panel, and Data tables.

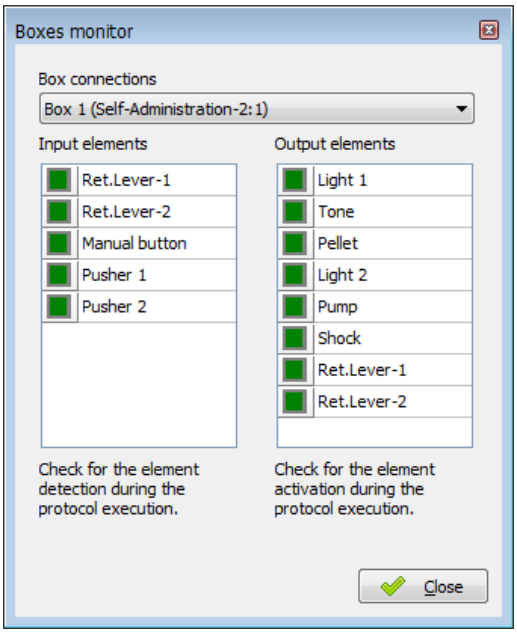


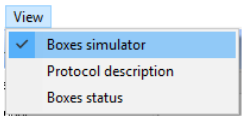
8.4.1.1 Run-Time panel main menu

The **Exit** option of the **File** menu closes the **Run Protocols** window.



The **Boxes monitor** option of the **View** menu opens the **Boxes monitor** panel for visualizing the activation and deactivation status of the different elements of the experimental box during the current session.





When the system is used with virtual boxes, the **Box monitor** option is replaced by the **Box simulator** option allowing the user to act on the **Box simulator** panel in an interactive way. In the Input element section, the user can activate or deactivate the

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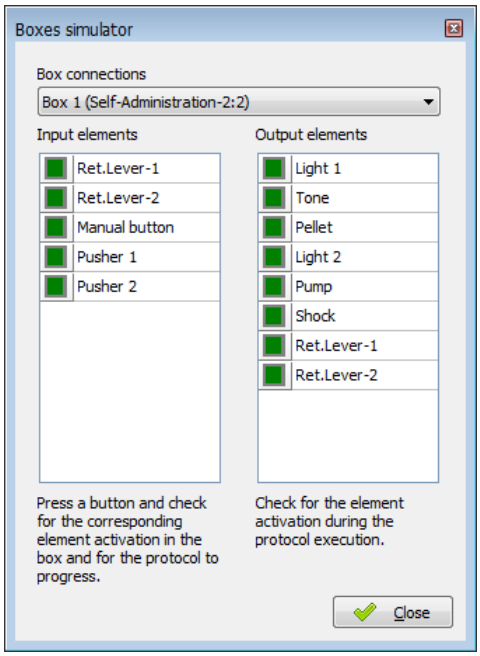
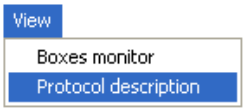
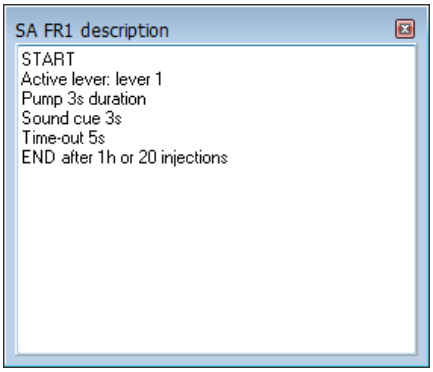
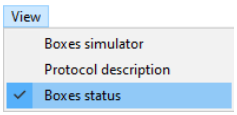
available elements (lever, button, pusher) and check whether the outputs' elements are changed accordingly. This option is very useful for checking the correct configuration of the protocols before running the real experiment.



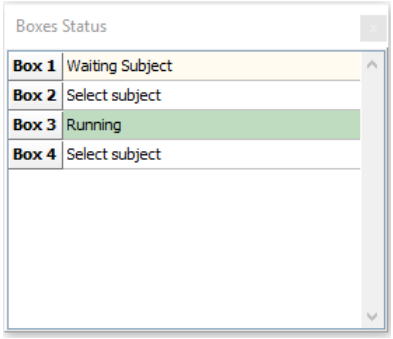


The **Protocol description** option of the **View** menu opens the protocol description associated with the protocol of the current box, as a reminder of the structure of the executed protocol.





The **Boxes status** option of the **View** menu opens Boxes Status panel that provides a global view of the status of all boxes:



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- **Select Subject**: means that the subject or subject list associated with this box is empty and the acquisition cannot be started. The user has to press the Select Subject button and associate subject(s) to this box.
- **Waiting Subject**: means that the box is ready to start the acquisition. The application is waiting for the user to press the start button, to put the subject onto the box , and then press the OK button to confirm the starting of the session.
- **Running**: means that data acquisition is running in this box. Once, the session is finished, the status would come back to the **Select Subject** or **Waiting Subject** status.
-

8.4.1.2 Run-Time panel tool bar

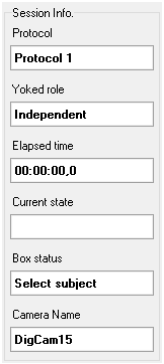
The options of the **Run** Menu are also available as a button of the Tool bar.

The **Select Subjects…** option of the Run menu allows selecting the subjects to be used in each experimental box.

The **Select Protocol…** option of the Run menu allows changing the protocol used in the current experimental box.

The **Run**, **Stop**, **Run All** and **Stop All** buttons of the Run menu allows controlling the initialization and ending of the sessions.

8.4.1.3 Run-Time panel Session Info



The Session Info panel provides some information about the evolution of the current session run in each box: the protocol executed, the linked role of the box, the current state of the protocol, the elapsing time from the beginning of the session, and the name of the camera related with the chamber (see section [0] for more information).

8.4.1.4 Run-Time panel data tables

The **Run protocols** window has a tab for every chamber to be run. In each tab, information about the status of the protocol is shown together with the important parameters of the execution.

The tables (States, Transitions, Subjects List tables) presented on the screen are the same ones that have been selected and configured in the **Run-Time panel configuration** section.

The different elements of the screen can be manually moved, increase/decrease in height and width. See next example:

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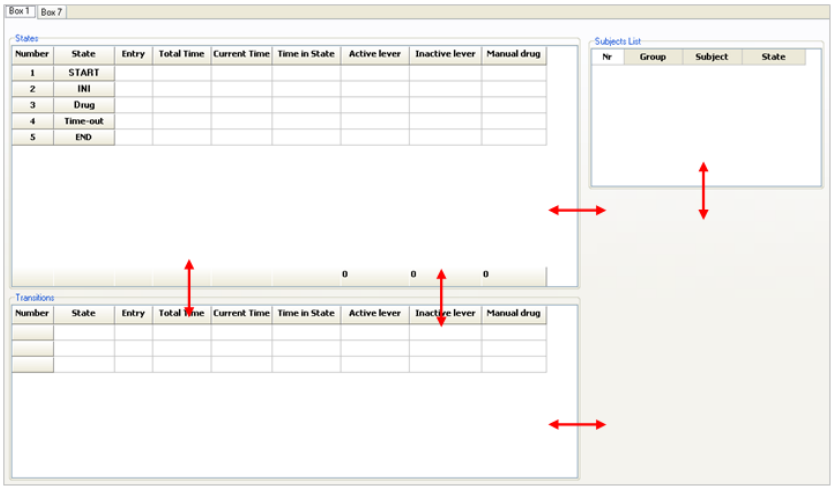


8.4.1.5 Start the acquisition procedure

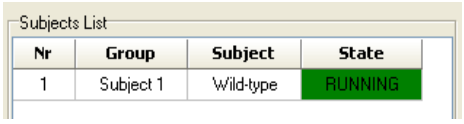
Once subjects are associated with boxes, the protocol can be started. Each protocol with its own independent linked role can be ran independently from the rest of the selected boxes.

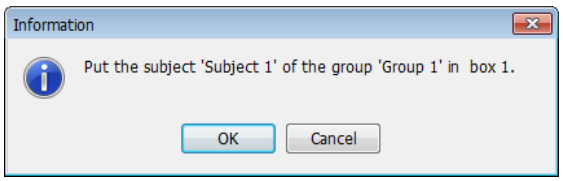
The boxes marked with slave yoked role can only start the acquisition by starting the execution of its related master box. Therefore, each couple of boxes related in a yoke procedure starts and finalizes the acquisition in synchronous manner.

Go to the item **Run** in the Main Menu and click on the option **Run** to start the execution of the current experimental chamber or **Run All** to start all the chambers at once. Or press the corresponding buttons in the tool bar.

The application will automatically select the first animal in the list of chosen subjects to start the trial.

The subject will appears as **RUNNING** in the **Subject List** and an **Information** message will appear.





Put every subject into its respective cage and press **OK**.

When the sessions are running, the experimenter can manually stop any of them by pressing the **Stop** or **Stop All** buttons.

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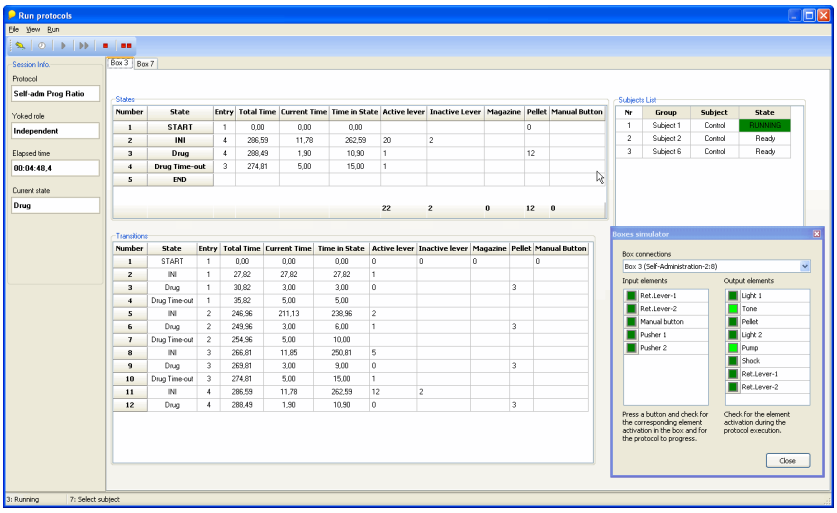
The **Stop** button will stop the current session. If the selected box is marked as master yoked then stopping the master will also stop the associated slave box. The **Stop All** button will simultaneously stop the sessions running in all the boxes. The user can also press the corresponding buttons in the tool bar.

8.4.1.6 On-screen Results

During the acquisition any event occurring in the cages will be registered.

The data acquisition main windows will provide current information about the running protocols, the current state of the elements in the experimental boxes, and the current data registered.



The Raw Data Tables (***Transitions*** and ***State***) will then give (in real-time) the parameters configured during the editing of the protocol or related to the Assistant tool used for configuring the protocol. The data will be continuously shown until the end of the experiment.

The **Session Info.** Section displays:

- the name of the **Protocol** running in the current experimental chamber
- the **Yoked Role** of the box.
- the name of the **Current state.**
- and the **Elapsed time** (hh:mm:ss.o) from the beginning of the experiment.

ON-SCREEN RESULTS PROVIDED BY THE PACKWINHO MODULE

The States and Transitions tables displayed in the Magazine & Hole training and 5-CSRT Run-Time panels are already preconfigured. Here is the description of the internal configurations made.

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Notes:

The Holes columns will only display the holes that have been selected in the Hole LED activation section of the Assistants.

The Total Detections and Manual Button are only present in the Run-Time panel associated to the Nine Hole box type (not for the Nine Hole Nine Pellets box type).

The content of the Run-Time panel configurations can be changed only if the Customized experimental module (PACKWINCS) has been purchased.

State table configuration

Data Columns	Updated by	Element	Value to put	Element
State	Change of State		Present State	
Entry	Change of State		Entries in State	
Total Time	Always		Time in Experiment	
Current Time	Always		Time in State	
Time in State	Always		Total Time in State	
Hole 1	Input Element Change	Hole 1	Total response in state	Hole 1
Hole 2	Input Element Change	Hole 2	Total response in state	Hole 2
Hole 3	Input Element Change	Hole 3	Total response in state	Hole 3
Hole 4	Input Element Change	Hole 4	Total response in state	Hole 4
Hole 5	Input Element Change	Hole 5	Total response in state	Hole 5
Hole 6	Input Element Change	Hole 6	Total response in state	Hole 6
Hole 7	Input Element Change	Hole 7	Total response in state	Hole 7
Hole 8	Input Element Change	Hole 8	Total response in state	Hole 8
Hole 9	Input Element Change	Hole 9	Total response in state	Hole 9
Reinforcement	Give Reinforcement	Pellet/Drop	Total Reinforcement in states	Pellet/Drop
Total Detections	Input Element Change	Detector	Total response in state	Detector
Manual button	Input Element Change	Manual button	Total response in state	Manual button

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Transition table configuration

Data Columns	Updated by	Element	Value to put	Element
State	Change of State		Present State	
Entry	Change of State		Entries in State	
Total Time	Always		Time in Experiment	
Current Time	Always		Time in State	
Time in State	Always		Total Time in State	
Hole 1	Input Element Change	Hole 1	Response in state	Hole 1
Hole 2	Input Element Change	Hole 2	Response in state	Hole 2
Hole 3	Input Element Change	Hole 3	Response in state	Hole 3
Hole 4	Input Element Change	Hole 4	Response in state	Hole 4
Hole 5	Input Element Change	Hole 5	Response in state	Hole 5
Hole 6	Input Element Change	Hole 6	Response in state	Hole 6
Hole 7	Input Element Change	Hole 7	Response in state	Hole 7
Hole 8	Input Element Change	Hole 8	Response in state	Hole 8
Hole 9	Input Element Change	Hole 9	Response in state	Hole 9
Reinforcement	Give Reinforcement	Pellet/Drop	Given pellets	Pellet/Drop
Total Detections	Input Element Change	Detector	Response in state	Detector
Manual button	Input Element Change	Manual button	Response in state	Manual button

ON-SCREEN RESULTS PROVIDED BY THE PACKWINVT MODULE

The States and Transitions tables displayed in the Pre-Test and Vogel Test Run-Time panels are already preconfigured. Here is the description of the internal configurations made. These configurations can be changed only if the user has purchased the PACKWIN Customized experimental module (CS).

State table configuration

Data Columns	Updated by	Element	Value to put	Element
State	Change of State		Present State	
Entry	Change of State		Entries in State	
Total Time	Always		Time in Experiment	
Current Time	Always		Time in State	
Time in State	Always		Total Time in State	
Licks	Input Element Change	Lickometer	Total response in state	Lickometer

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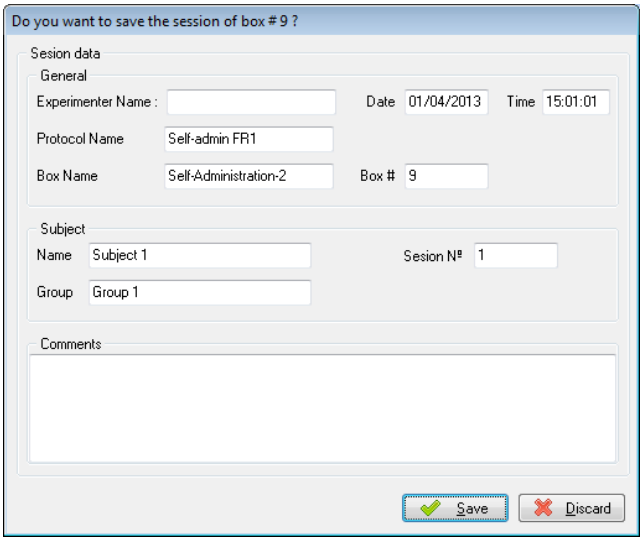
Transition table configuration

Data Columns	Updated by	Element	Value to put	Element
State	Change of State		Present State	
Entry	Change of State		Entries in State	
Total Time	Always		Time in Experiment	
Current Time	Always		Time in State	
Time in State	Always		Total Time in State	
Licks	Input Element Change	Lickometer	Response in state	Lickometer

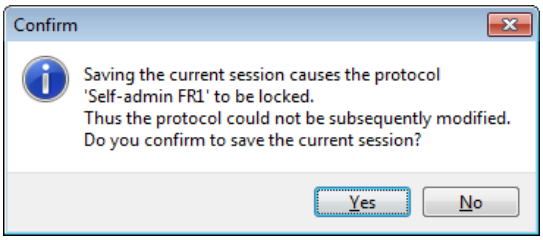
8.4.1.7 Save data

Once the acquisition process is finished in one box, the following window appears.



The Section **Comments** can be edited by the user.

When performing the protocol for the first time within the experiment file, clicking on the **Save** button pops a message up indicating that the associated protocol will appear as locked in the Protocols organizer table.



Note: In boxes involved in the yoked procedure, the saving panel is only shown for the Master boxes. The Comments entered here will be saved for both yoked boxes.

8.4.1.8 Export the data to Excel

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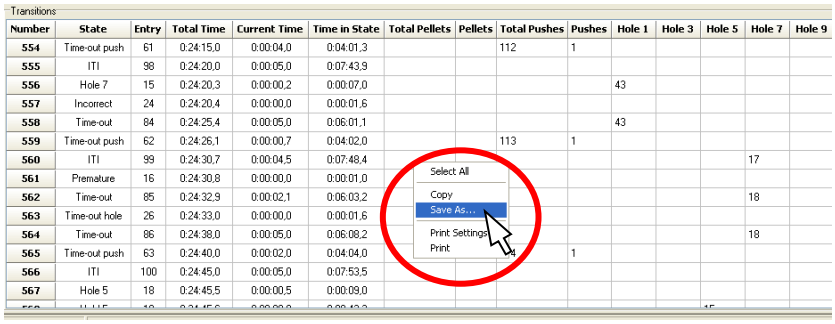
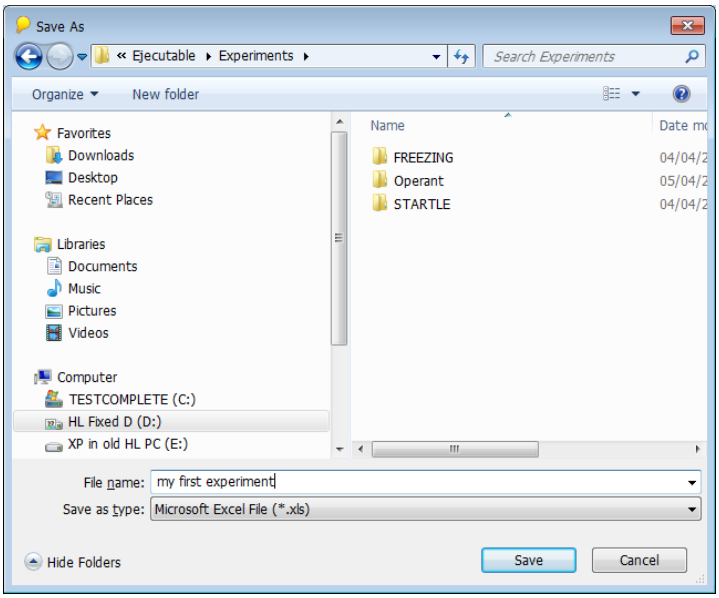
The States and Transition tables obtained during the acquisition can be exported to Excel, HTML, CSV, or Word file.



Right click on the table, select **Save as**, enter the name of the file, select the file format (can be Excel, HTML, CSV, or Word file), and press **save.**



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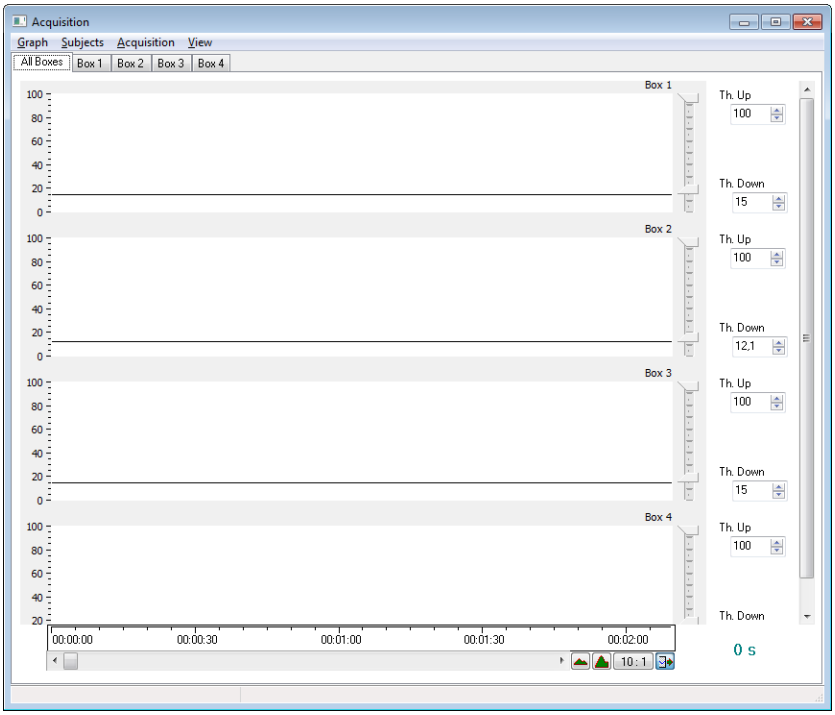
8.4.2 PACKWIN Freezing and Startle Module (CSFR and CSST)

8.4.2.1 Run-Time Panel

The Startle/Freezing Run-Time panel contains 2 box views.

ALL BOXES VIEW

The ***All boxes*** tab shows the analogic signals of animal activity registered from the load cells for all the boxes that have been previously selected in the **Select protocols and boxes to run panel** (see chapter 8.2)



A vertical scroll bar is provided to access all the presented plots.

INDIVIDUAL BOX VIEW

Additional tabs are provided for an individual view of the data acquisition process in each selected box.

The acquisition individual view panels show the following information:

(1) the analogic signal plot (signal = f(time)) showing the signal of activity registered from the load cells,

(2) two adjustable activity thresholds,

(3) the current subject section,

(4) a raw data table,

(5) a summary data table.

(6) **camera availability**

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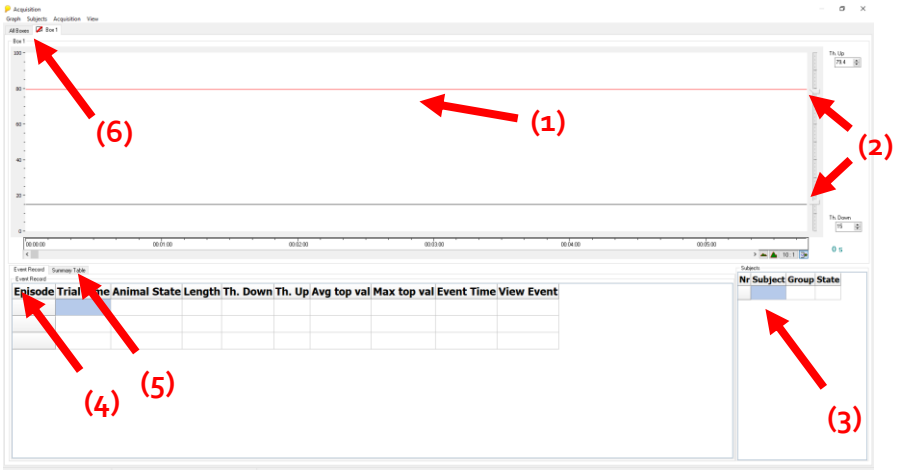
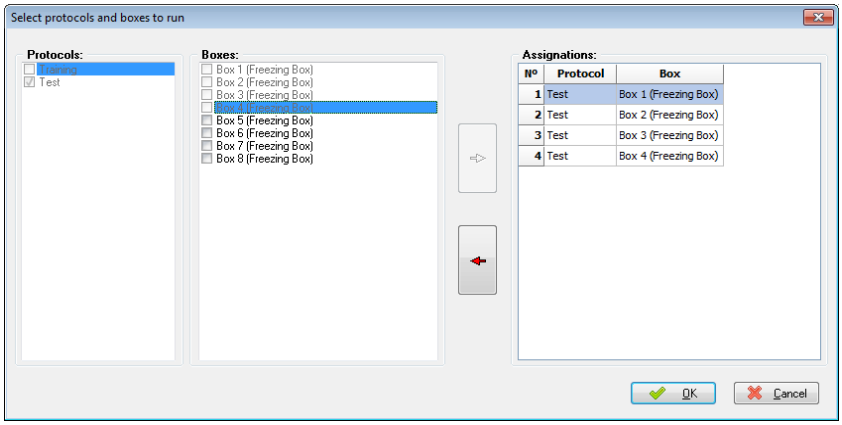


8.4.2.2 Associate Subjects and Boxes

To select the subject/subjects that will be used in each box, select the S**elect Subjects...** option of the **Subjects** menu.

The panel used for selecting the subjects that will be used in each box is similar to the one used for assigning the protocols to the boxes.



To associate subjects and boxes:

- Select a box in the **Boxes Tree** panel.
- Select a subject, several subjects or a list of subjects from the **Groups of Subjects** or **Lists o Subjects** tabs.
- Associate or de-associate the box and the subject by pressing the corresponding right or left arrow buttons.
- Press the **OK** button when done.

In the Run protocol screen, the subject will appear as **Ready** in the **Subject List** table (more than one animal per box can be selected).

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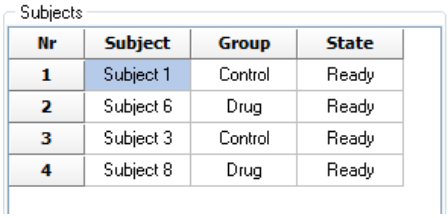
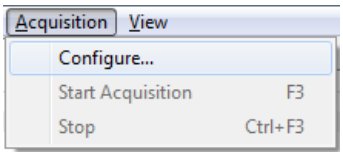


If two or more boxes are used, a subject (or a list of subjects) has to be selected for each box. The acquisition of data will not be allowed for the boxes without subject (START ACQUISITION options will be shown as disabled).

8.4.2.3 Configure the Session

Select the **Configure…** option in the **Acquisition** menu.



SET THE GAIN

Gain configuration

The gain is a parameter that controls the amplification for the activity signal received from the load cells. If the signal is low, the amplification has to be high, and on the contrary, if the signal is too high, the amplification has to be lowered.

The gain is set from the Unit for Load Cell Coupler (LE 111) and can be fine-tuned here with the software. The gain can be changed depending on the amplitude of the signal visualized (it has to be checked in preliminary trials before starting a data acquisition session that you intend to scientifically evaluate).

For those systems migrating from PCI card to new USB connections: USB-based systems provide signals with exactly half of the amplitude given by PCI-based systems. In this case, if you have executed experiments in a PCI-based system and you want to continue them in a USB-based system, the gain selected within the software MUST be doubled (e.g., a gain of 4 in PCI must be set to 8 in USB).

When the system is used in Virtual box mode (simulator), the only value available in the Gain Control section is "1".

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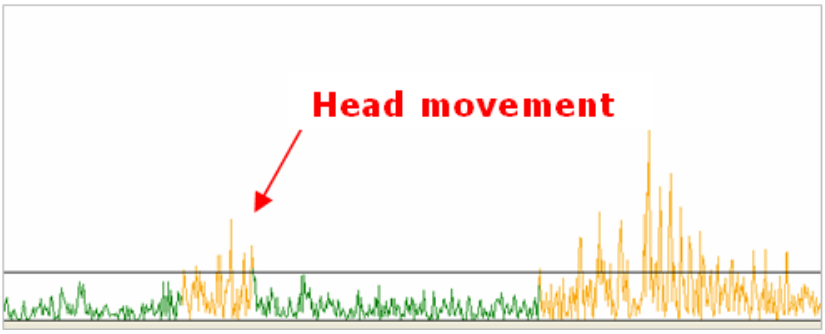
How to choose the value of the GAIN for the whole system (for LE111 unit + software) is a recurrent question among the users of the system. Please find some recommendations in the next two chapters.

How to choose the value of the gain for fear conditioning experiments

The gain setting is crucial for obtaining a correct detection of the subject movements and by consequence for enabling the distinction between animal immobility related to freezing from animal activity (locomotion, grooming, rearing, head movements...). It is then very important to set the gain high enough to detect small movements, such as the head movements (see example in the next figure).



GAIN recommendations for the LE111 device: 1000-2000 for 250g-300g rats and 5000 for mice.

GAIN recommendations from the software: we do not have standard value recommendations for setting the gain from the software, as it depends also on your animals. Make sure that any animal head movement is detected by the system by producing an activity event on the analogic signal visualized on the screen. In a general manner, the higher gain value is preferred when working with mice. Choose the 1:1 zoom graph setting on the screen in order to see better the changes in the signal induced by the animal movements.

How to choose the value of the gain for startle reflex experiments (like PPI experiments) experiments

In Startle experiments, the gain has to be chosen in order to show in the plot the overall amplitude of the startle reflex in responses to all the stimuli or combination of stimuli used in the protocol.

As an example, in Prepulse Inhibition of Startle-Reflex experiments (PPI), the amplitude of the basal pulse startle response has to be high enough to allow the evaluation of an inhibition of the response when combined with the prepulse; on the other hand, it has to be NOT TOO HIGH to avoid saturation (amplitude>100%) of the response (see next figure).

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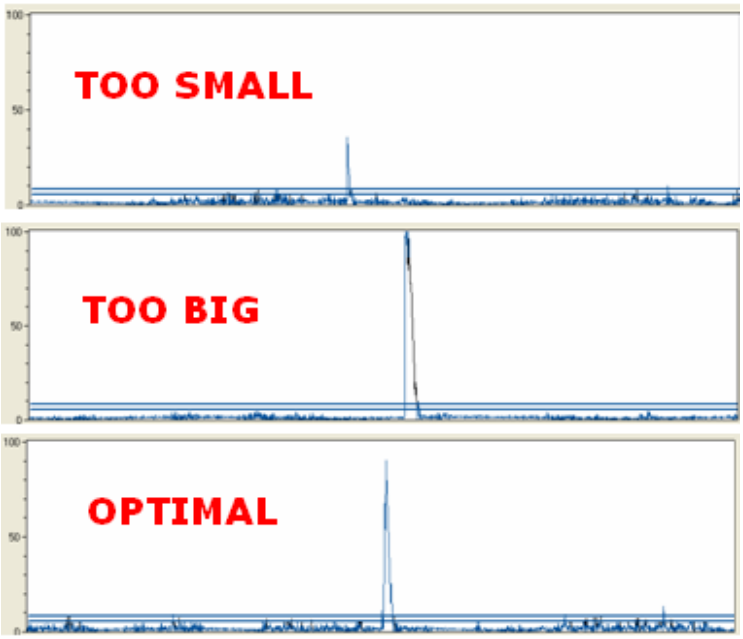


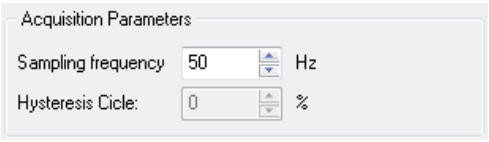
GAIN recommendations for the LE111 device: 1000-2000 for 250g-300g rats and 5000 for mice.

GAIN recommendations from the software: as with freezing experiment, we do not have standard value recommendations for setting the gain from the software as it depends on the animals. Pilot studies have to be performed in order to determine the optimal GAIN value that can be used with all the animals of the same experiment.

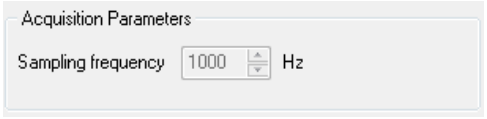
SAMPLING FREQUENCY

The **Sampling frequency** corresponds to the sampling frequency for data acquisition.

The value of the sampling frequency in the **FREEZING** module is set by default to 50 Hz. We recommend the user to not change these values.



Value of the sampling frequency is set by default to 1000 Hz in the **STARTLE** module and cannot be modified by the user.



FREEZING CRITERION (ONLY FOR PACKWIN-CSFR MODULE)

Freezing behavior is commonly defined as the complete immobilization of the animal except for respiratory movements. In Fear Conditioning systems, the

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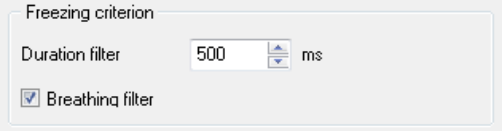
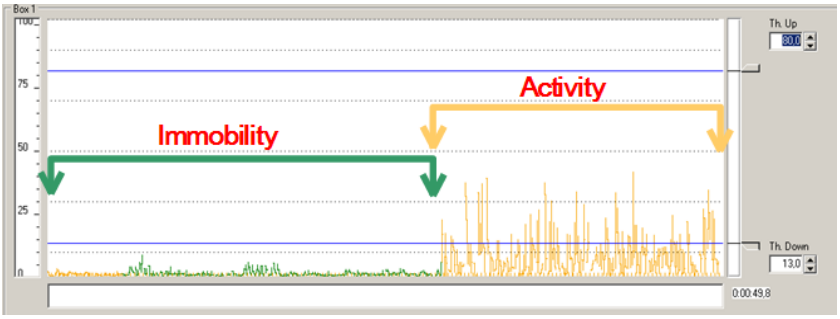
freezing episodes are mainly related to the detection of the subject immobility. Three criteria/settings ensure a reliable detection of Freezing/immobility episodes: rough immobility detection is ensured by the **Low activity threshold** (1) available from the main acquisition panel and fine adjustments of freezing detection are ensured by the **Duration filter** (2) and the **Breathing filter** (3) available from the present configuration panel.



These three settings are applied on the signal registered during acquisition. They can be set before the acquisition of the signal and changed afterward during the analysis process to obtain a new set of data.

Low activity threshold

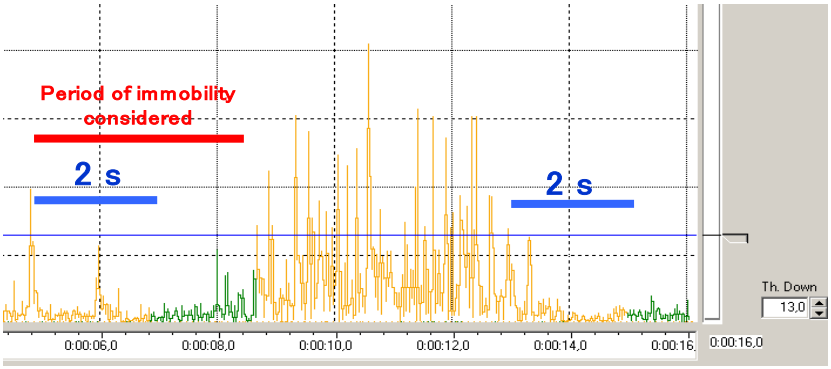
The subject is considered as immobile when the amplitude of the signal (activity value) is under the user-defined Low activity threshold (Th. Down).



Duration filter

The duration filter corresponds to the period of time in which the activity signal has to be under the threshold, thus the animal will be considered as immobile. This option allows excluding non-specific freezing episodes.

In the next example, the Duration filter is of 2 s. The program will only consider the animal as immobile when the activity is under the threshold activity for at least 2 s. All periods of inactivity with duration lower than 2 s will not be taken into account.



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Breathing filter

When a breathing episode is detected during an immobility episode thanks to the breathing filter, it is ignored by the system, so the immobility detection is not disrupted.

If the breathing filter is not activated, most of the breathing episodes detected would be considered as activity, so the actual time spent in freezing would be underestimated.

Fig. A: example of signal without breathing filter (duration filter = 1 s) – the breathing episodes disrupt the detection of the immobility/freezing. Orange colour means Activity; green colour means Immobility.

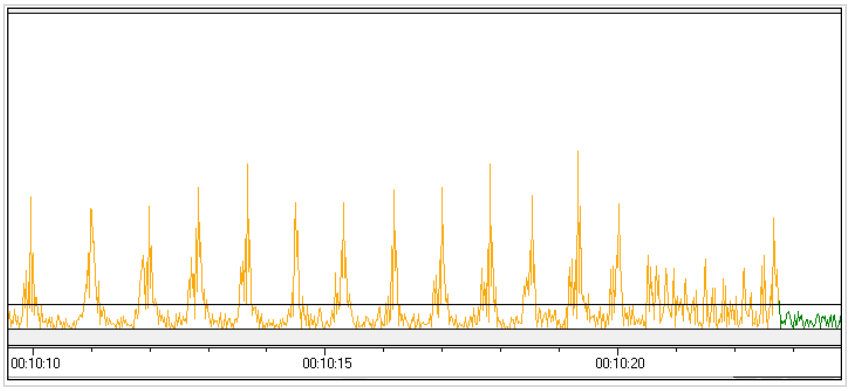
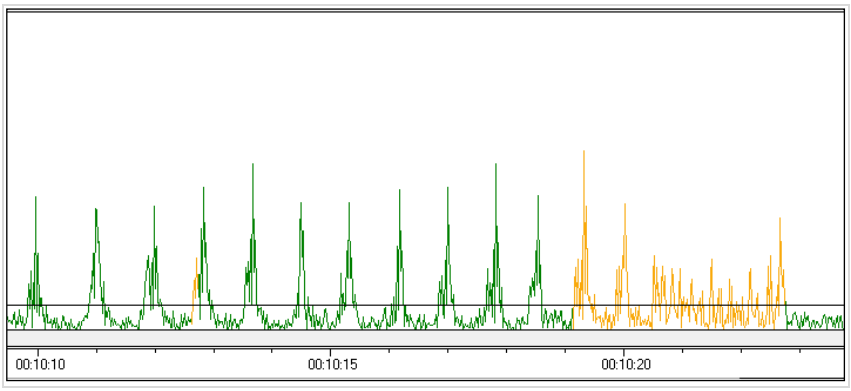


Fig. B: the same signal with breathing filter – the system is ignoring the breathing episode and makes more reliable the detection of the freezing behavior.



The criterion for immobility/freezing may vary between animal species, animal weight, experimenters, and laboratories. We strongly recommend the user perform pilot studies comparing the values obtained automatically by the system, with those obtained manually through the users hand scoring and visual observations. After the user has identified the ideal conditions/settings for their project goal, they should apply the same configurations to all the experiments for that goal.

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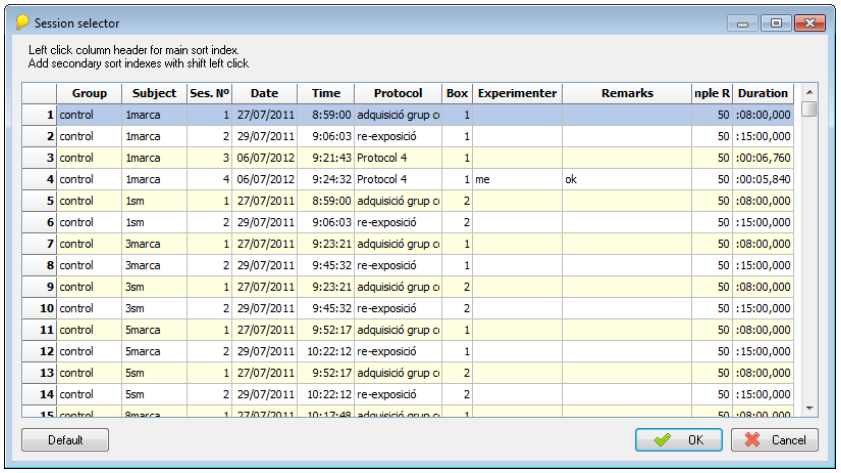
DEMO SESSION

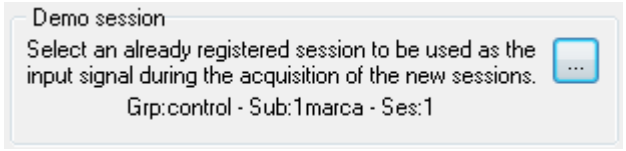
PACKWIN platform provides the possibility to run again the acquisition of a load cell signal from a session registered in the experimental file.

- Press the button to open the available list of sessions registered in the experimental file.



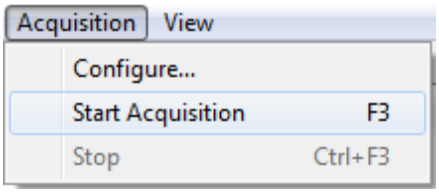
	Group	Subject	Ses. Nº	Date	Time	Protocol	Box	Experimenter	Remarks	nple R	Duration
1	control	1marca	1	27/07/2011	8:59:00	adquisició grup c	1			50	:08:00,000
2	control	1marca	2	29/07/2011	9:06:03	re-exposició	1			50	:15:00,000
3	control	1marca	3	06/07/2012	9:21:43	Protocol 4	1			50	:00:06,760
4	control	1marca	4	06/07/2012	9:24:32	Protocol 4	1	me	ok	50	:00:05,840
5	control	1sm	1	27/07/2011	8:59:00	adquisició grup c	2			50	:08:00,000
6	control	1sm	2	29/07/2011	9:06:03	re-exposició	2			50	:15:00,000
7	control	3marca	1	27/07/2011	9:23:21	adquisició grup c	1			50	:08:00,000
8	control	3marca	2	29/07/2011	9:45:32	re-exposició	1			50	:15:00,000
9	control	3sm	1	27/07/2011	9:23:21	adquisició grup c	2			50	:08:00,000
10	control	3sm	2	29/07/2011	9:45:32	re-exposició	2			50	:15:00,000
11	control	5marca	1	27/07/2011	9:52:17	adquisició grup c	1			50	:08:00,000
12	control	5marca	2	29/07/2011	10:22:12	re-exposició	1			50	:15:00,000
13	control	5sm	1	27/07/2011	9:52:17	adquisició grup c	2			50	:08:00,000
14	control	5sm	2	29/07/2011	10:22:12	re-exposició	2			50	:15:00,000

- Select the session to be acquired again (highlighted in clear blue) and press the OK button.
- The name of the selected session will appear in the **Demo session** section.



8.4.2.4 Start the Acquisition Procedure

When the subjects have been selected the protocol can be run. Select the **Start Acquisition** option in the **Acquisition** menu or press the **F3** key.



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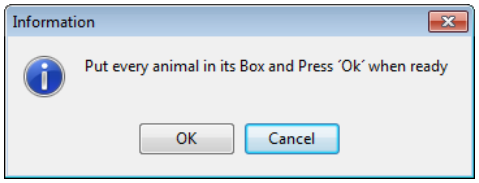
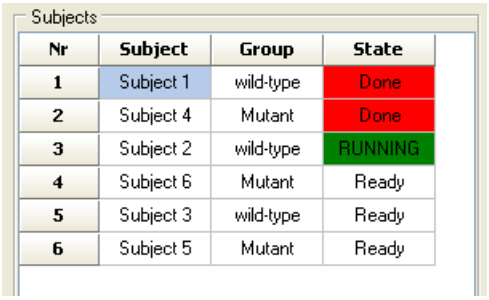
Put every animal into its respective box and press ***OK***



All the boxes will start synchronically and the acquisition of the activity signal from the load cells will begin, registering the signal of the animal activity in the boxes.



During the acquisition process, the **Subjects** table summarizes the subjects that will be experimented in the current box, as well as their current state:

- ***Done*** subject which has performed the experiment.

- ***Waiting***: subject to put in the cage.

- ***Running***: subject performing the experiment.

- ***Ready***: next subjects.

The experimenter can stop the experiment at any time of the acquisition process. Select the **Stop** option in the **Acquisition** menu or press the **Ctrl + F3** key combination to end the session on all chambers.

8.4.2.5 On-screen Results

The Acquisition screen displays the analogic signal received for the weight transducers and the data table calculated in real-time.

LOAD CELL ANALOGICICAL SIGNAL

Freezing analogic signal (PACKWIN-CSFR)

The signal corresponding to the subject activity can be visualized in real-time.

A unique X axis shows the session acquisition time (hh:mm:ss). Y axis represents the measured activity in % of the maximal capacity of the load cell system (100%).

This is an example of results obtained with a mouse of 20 g (LE 111 unit, FILTER 0.5Hz, GAIN 5000.)

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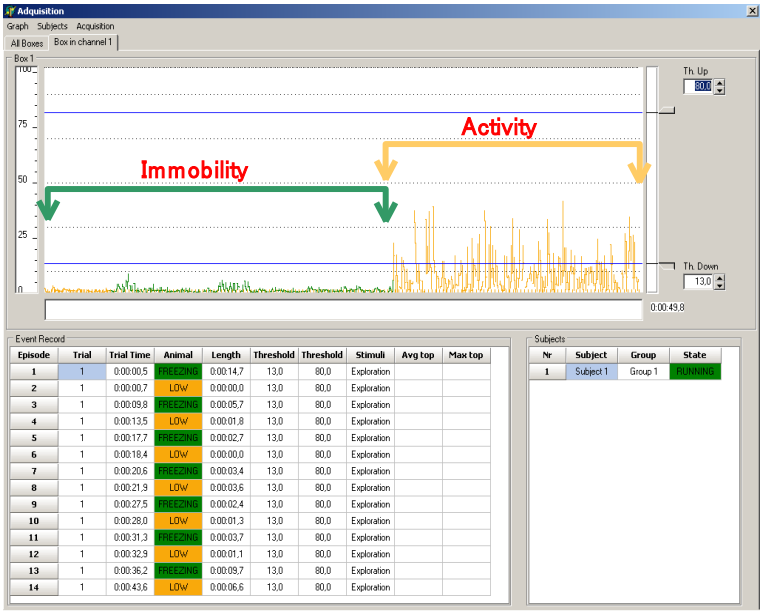
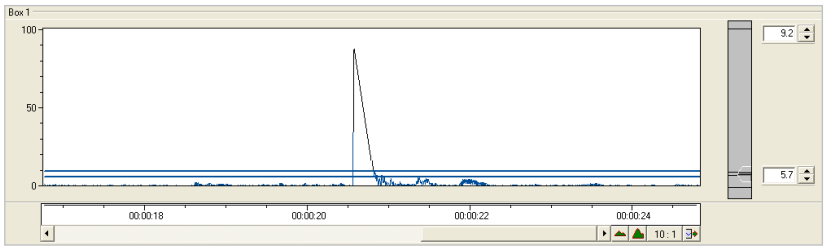


The colour displayed on the signal indicates which portion of the signal is considered as an immobile/freezing episode (green colour), a low activity episode (orange/yellow) or a high activity episode (red).

The colour of the signal and data provided by the numerical table depends on the freezing criteria defined by the user (Low activity threshold, Duration filter and Breathing filter).

Startle analogic signal (PACKWIN-CSST)

The signal corresponding to the startle response is displayed in real-time. In Startle Reflex systems the analogic signal is smoothed for optimizing the calculation of startle reflex duration, latency to onset, and average.



A unique X axis shows the session acquisition time (hh:mm:ss). Y axis represents the measured activity in % of the maximal capacity of the load cell system (100%).

Zoom

The X-time axis is provided of a zoom-in and zoom-out options, using respectively the and buttons located at the bottom right side of the signal. The zoom list can be also used.

Zoom is only applied in the time scale (X axis).

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Horizontal scroll

The horizontal scroll is used by displacing the horizontal scroll bar located just below X-time axis.

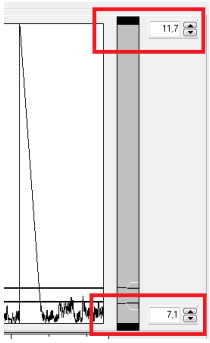
Automatic horizontal scroll (plot following) can be done by pressing the button located at the end of the horizontal scroll bar.

Activity threshold

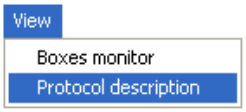
Two activity thresholds available on the right section of the signal plot can be set for discriminating Immobility episodes from Low Activity and High activity episodes.

In the PACKWIN-CSFR Freezing module, the Low Activity threshold (Th.Down) discriminates the immobility episodes (freezing) from the activity episodes and the High Activity threshold (Th. Up) discriminates the Low Activity episodes from the High Activity episodes.

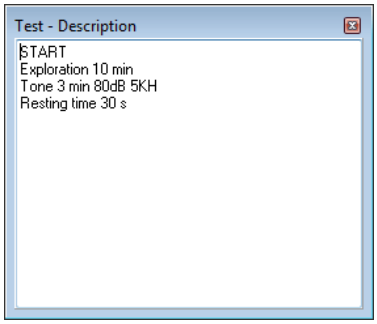
In the PACKWIN-CSST Startle module, the two activity thresholds available on the right section of the signal plot can be set for determining the START and END of each startle response.

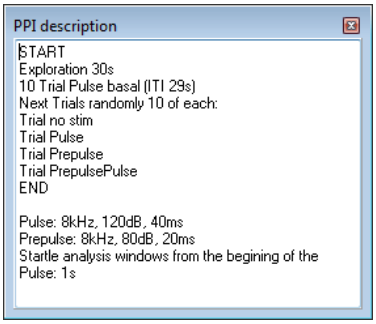


PROTOCOL DESCRIPTION



The **Protocol description** option of the **View** menu opens the protocol description associated with the protocol of the current box, as a reminder of the structure of the executed protocol.





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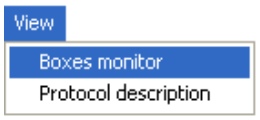
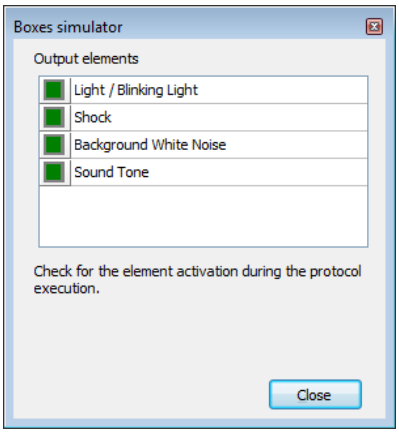
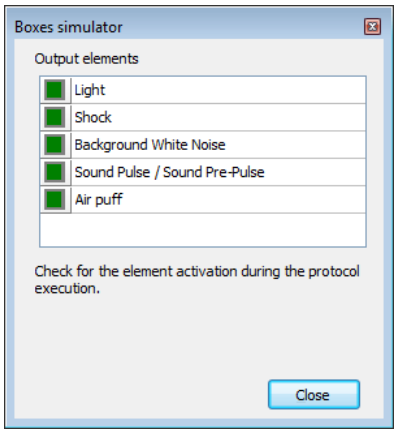
BOX MONITOR

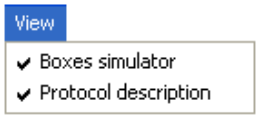


The **Boxes monitor** option of the **View** menu opens the **Boxes monitor** panel for visualizing the activation and deactivation status of the different elements of the experimental box during the current session.





FREEZING module STARTLE module



When the system is used with virtual boxes, the **Box monitor** option is replaced by the **Box simulator** option allowing the user to act on the **Box simulator** panel in an interactive way.

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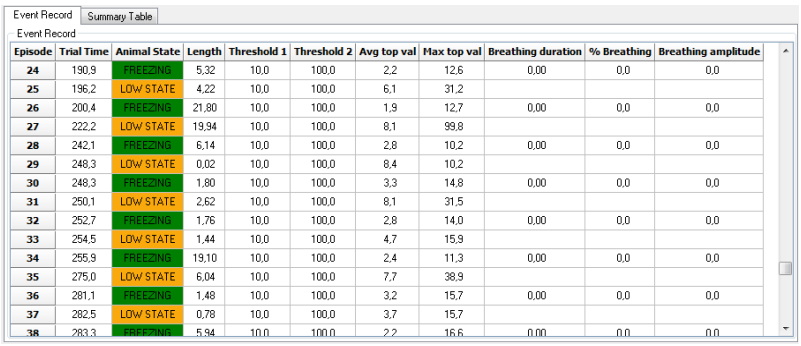
DATA TABLES PROVIDED BY THE PACKWIN-CSFR MODULE

Event record data table

The Event Record Table of the FREEZING module provides the list of the distribution of the immobility and activity episodes detected along the time until the end of the acquisition process.

Column title	Description
: Episode	trial number
Trial Time	time in the experiment (sec)
Animal State	animal activity: FREEZING (<low threshold), LOW movements (low<<high threshold), HIGH movements (>high threshold)
Length	Duration of the current episodes of FREEZING, LOW and HIGH movements,
Th.Down	Low activity Threshold set in the graph
Th. Up	High activity Threshold set in the graph
Avg top val	Average amplitude of the signal registered during the current episode.
Max top val	Maximum amplitude of the signal registered during the current episode.
Breathing duration	Total duration (in seconds) of episodes of breathing during freezing episode. Only available if the "Breathing filter" is active.
% Breathing	Percentage of breathing detected during the current freezing episode: breathing duration x 100/ Freezing Length. Only available if the "Breathing filter" is active.
Breathing amplitude	Average maximum amplitude of all breathing signal detected during the current freezing episode. Only available if the "Breathing filter" is active.


Event Record | Summary Table

Event Record

Episode	Trial Time	Animal State	Length	Threshold 1	Threshold 2	Avg top val	Max top val	Breathing duration	% Breathing	Breathing amplitude
24	190,9	FREEZING	5,32	10,0	100,0	2,2	12,6	0,00	0,0	0,0
25	196,2	LOW STATE	4,22	10,0	100,0	6,1	31,2			
26	200,4	FREEZING	21,80	10,0	100,0	1,9	12,7	0,00	0,0	0,0
27	222,2	LOW STATE	19,94	10,0	100,0	8,1	99,8			
28	242,1	FREEZING	6,14	10,0	100,0	2,8	10,2	0,00	0,0	0,0
29	248,3	LOW STATE	0,02	10,0	100,0	8,4	10,2			
30	248,3	FREEZING	1,80	10,0	100,0	3,3	14,8	0,00	0,0	0,0
31	250,1	LOW STATE	2,62	10,0	100,0	8,1	31,5			
32	252,7	FREEZING	1,76	10,0	100,0	2,8	14,0	0,00	0,0	0,0
33	254,5	LOW STATE	1,44	10,0	100,0	4,7	15,9			
34	255,9	FREEZING	19,10	10,0	100,0	2,4	11,3	0,00	0,0	0,0
35	275,0	LOW STATE	6,04	10,0	100,0	7,7	38,9			
36	281,1	FREEZING	1,48	10,0	100,0	3,2	15,7	0,00	0,0	0,0
37	282,5	LOW STATE	0,78	10,0	100,0	3,7	15,7			
38	283,3	FREEZING	5,94	10,0	100,0	2,2	16,6	0,00	0,0	0,0

A new line is added each time the system detects a new activity state.

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Summary data table

The Summary table gives in real-time the following parameters for each state of the protocol:

Column title	Description
State	name of the state
Freezing duration	accumulated freezing time.
Activity duration	accumulated activity time.
% Freezing	percent of time in freezing.
% Activity	percent of time in activity.
Nr. Freezing Episodes	Number of Freezing episodes detected in this state.
Avg top val	Average amplitude of the signal registered.
Max top val	Maximum amplitude of the signal registered.
Breathing duration	Total duration (in seconds) of episodes of breathing during protocol state. Only available if the "Breathing filter" is active.
% Breathing	Percent of time the subject was breathing during the freezing episode. Percentage of breathing detected in the state: breathing duration x 100/ Freezing Duration. Only available if the "Breathing filter" is active.
Breathing amplitude	average maximum amplitude of all breathing signal detected in the state. Only available if the "Breathing filter" is active.

At the bottom line of the table, total values are displayed:

Column title	Description
Total Freezing duration	Total duration of Freezing detected during the session.
Total Activity duration.	Total duration of Activity detected during the session.
Total % Freezing.	Percentage of time the subject was in Freezing during the whole session.
Total % Activity.	Percentage of time the subject was in Activity during the whole session.
Total Freezing duration	Total duration of Freezing detected during the session.
Total Activity duration.	Total duration of Activity detected during the session.

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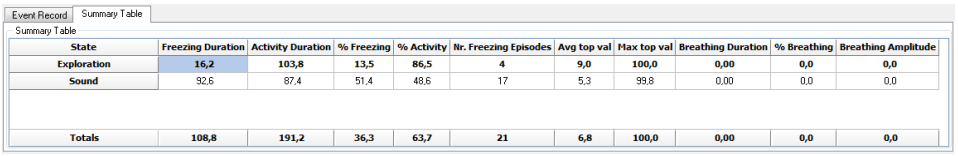


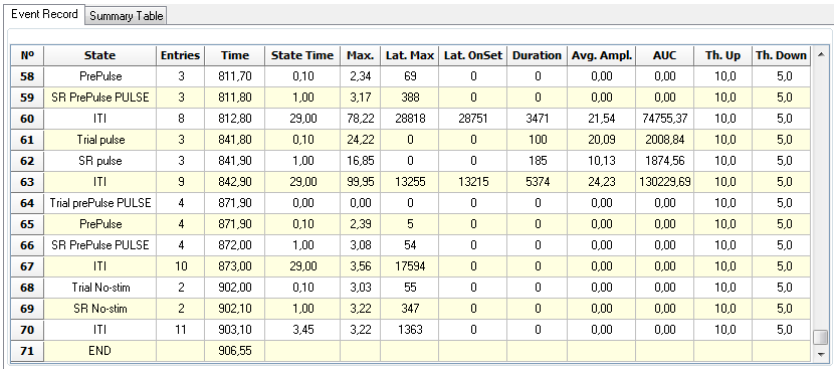
State	Freezing Duration	Activity Duration	% Freezing	% Activity	Nr. Freezing Episodes	Avg top val	Max top val	Breathing Duration	% Breathing	Breathing Amplitude
Exploration	16,2	103,8	13,5	86,5	4	9,0	100,0	0,00	0,0	0,0
Sound	92,6	87,4	51,4	48,6	17	5,3	99,8	0,00	0,0	0,0
Totals	108,8	191,2	36,3	63,7	21	6,8	100,0	0,00	0,0	0,0

DATA TABLES PROVIDED BY THE PACKWIN-CSST MODULE

Event record data table

The Event Record Table of the STARTLE module provides the next parameters shown in the table below for each state of the protocol.

Column title	Description
N°	State transition number
State	name of the current state
Entries	number of entries into the current state
Time	time in the experiment (sec) in which the state ends (during acquisition) / starts (during analysis)
State time	state duration (sec)
Max.	maximum amplitude of startle response (%)
Lat. Max	latency to achieve maximal startle response (msec)
Lat. Onset	latency to achieve the onset of startle response (msec)
Duration	duration of startle response (msec)
Avg. Ampl.	mean average value of the amplitude of startle response (arbitrary unit)
AUC	area under the curve of the startle response (arbitrary unit)
Th. Down	threshold to define the beginning of startle response (%)
Th. Up	threshold to define the end of startle response (%)



N°	State	Entries	Time	State Time	Max.	Lat. Max	Lat. OnSet	Duration	Avg. Ampl.	AUC	Th. Up	Th. Down
58	PrePulse	3	811,70	0,10	2,34	69	0	0	0,00	0,00	10,0	5,0
59	SR PrePulse PULSE	3	811,80	1,00	3,17	388	0	0	0,00	0,00	10,0	5,0
60	ITI	8	812,80	29,00	78,22	28818	28751	3471	21,54	74755,37	10,0	5,0
61	Trial pulse	3	841,80	0,10	24,22	0	0	100	20,09	2008,84	10,0	5,0
62	SR pulse	3	841,90	1,00	16,85	0	0	185	10,13	1874,56	10,0	5,0
63	ITI	9	842,90	29,00	99,95	13255	13215	5374	24,23	130229,69	10,0	5,0
64	Trial prePulse PULSE	4	871,90	0,00	0,00	0	0	0	0,00	0,00	10,0	5,0
65	PrePulse	4	871,90	0,10	2,39	5	0	0	0,00	0,00	10,0	5,0
66	SR PrePulse PULSE	4	872,00	1,00	3,08	54	0	0	0,00	0,00	10,0	5,0
67	ITI	10	873,00	29,00	3,56	17594	0	0	0,00	0,00	10,0	5,0
68	Trial No-stim	2	902,00	0,10	3,03	55	0	0	0,00	0,00	10,0	5,0
69	SR No-stim	2	902,10	1,00	3,22	347	0	0	0,00	0,00	10,0	5,0
70	ITI	11	903,10	3,45	3,22	1363	0	0	0,00	0,00	10,0	5,0
71	END		906,55									

A new line is added each time the protocol enters into a new state.

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Summary data table

The Summary table gives in real-time the next parameters for each state of the protocol:

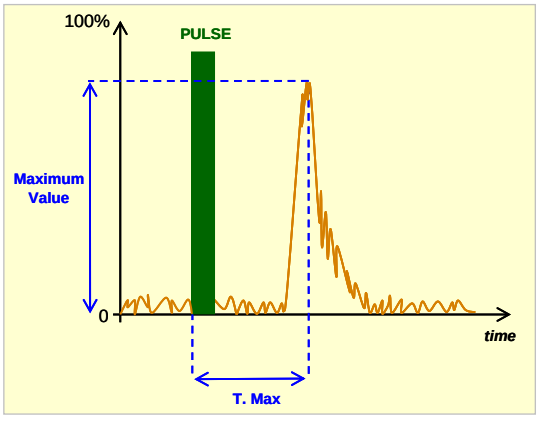
Column title	Description
State	name of the state.
Total Ent.	number of total entries into the state.
Mean Max.	Mean of maximum amplitude of startle response (%)
Mean Lat. Max	Mean of latency to achieve maximal startle response (msec)
Mean Lat. Onset	Mean of latency to achieve the onset of startle response (msec)
Mean Duration	Mean of duration of startle response (msec)
Mean Avg. Ampl	Mean average value of startle response amplitude (arbitrary unit)
Mean AUC	Mean area under the curve of startle response (arbitrary unit)

Event Record | Summary Table

State	Total Ent.	Mean Max.	Mean Lat. Max.	Mean Lat. Onset	Mean Duration	Mean Avg. Ampl.	Mean AUC
Trial pulse basal	11	9,35	37,91	0,00	9,09	6,09	608,83
SR pulse basal	10	12,45	456,70	0,00	79,30	5,39	4271,84
ITI basal	10	58,23	11360,20	5824,30	1293,50	25,99	50498,93
Trial No-stim	2	2,64	38,00	0,00	0,00	0,00	0,00
SR No-stim	2	3,05	402,00	0,00	0,00	0,00	0,00
Trial pulse	3	9,67	15,67	0,00	33,33	6,70	669,61
SR pulse	3	7,73	369,33	0,00	61,67	3,38	624,85
Trial prePulse	2	2,93	65,50	0,00	0,00	0,00	0,00
SR prePulse	2	3,03	570,50	0,00	0,00	0,00	0,00
Trial prePulse PULSE	4	0,00	0,00	0,00	0,00	0,00	0,00
PrePulse	4	2,59	49,75	0,00	0,00	0,00	0,00
SR PrePulse PULSE	4	3,11	325,00	0,00	0,00	0,00	0,00
ITI	11	43,80	13941,73	7471,64	1292,82	12,96	32207,38
Totals	69	21,59	4090,71	2103,67	466,45	8,78	16142,21

Activity threshold independent calculations

Two calculations are given **independently** of the choice of the two activity thresholds: the maximum amplitude of the startle response (Maximum value) and the latency to achieve maximal startle response (T.Max), see next figure.



The maximum amplitude of the startle response is considered as **the most discriminative parameter to evaluate the startle response** and it is generally the

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only parameter referenced in the literature. All the other parameters are given as additional/complementary parameters for the complete description of the startle response.

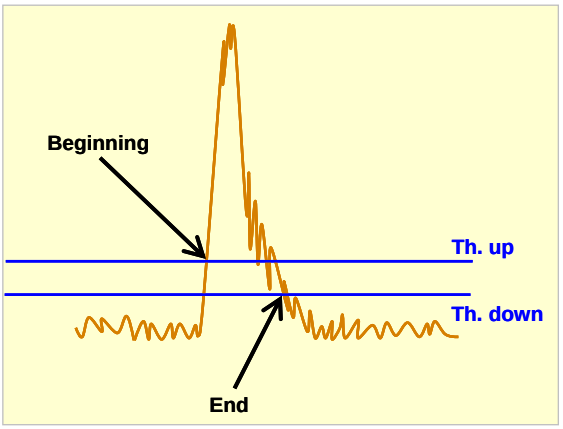
Activity threshold dependent calculations

Three of the given calculations are entirely **dependent** of the choice of the two activity thresholds, Th. Up and Th. Down: the duration of startle response (duration), the mean area value of the response (average) and the latency to achieve the beginning of the startle response (startle latency).

These two thresholds are necessary to define the beginning and the end of the startle response.

The startle response begins when the activity signal (orange/yellow) is higher than the up threshold (Th. up) and ends when the activity signal is lower than the down threshold (Th. down).

We recommend placing the up and down thresholds just above the animal's baseline activity level as shown in the next figure.



The 3 calculations cited above can be then determined as described in the next images:

- **Duration** and **Latency** of the startle response.

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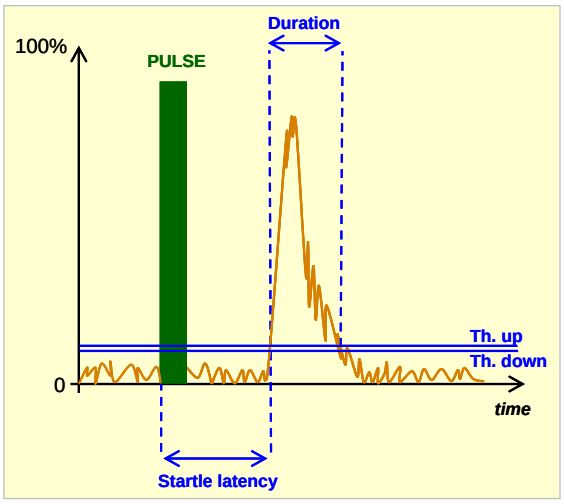
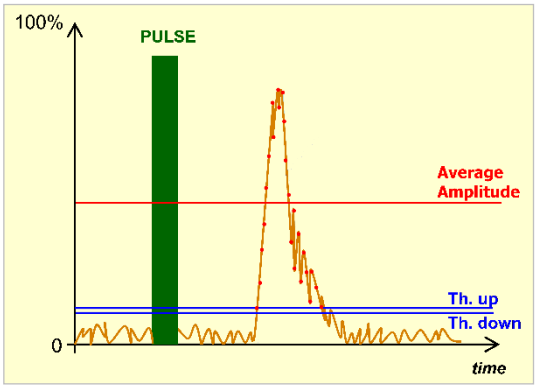


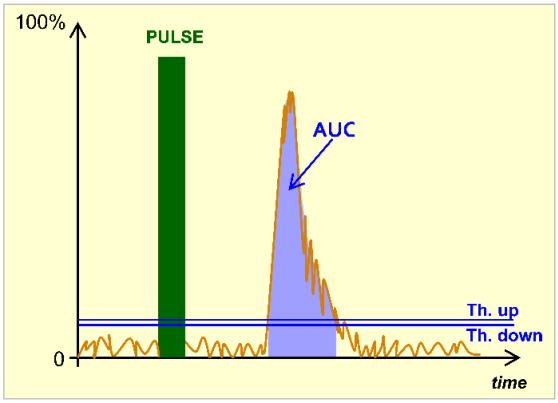
- **Avg. Ampl.**

The Avg. Ampl. (average amplitude) is the mean of the amplitude values of the signal comprised between the beginning and the end of the startle response.



- **AUC**

The AUC (area under the curve) represents the area between the signal and the X axis comprised between the beginning and the end of the startle response.



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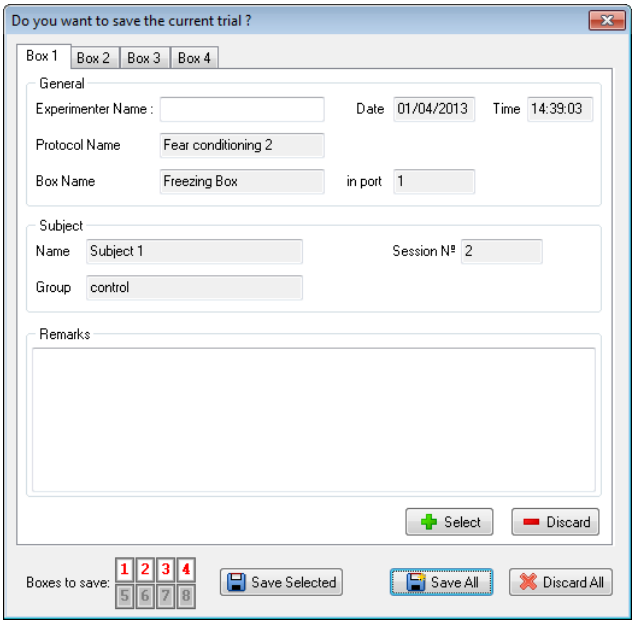
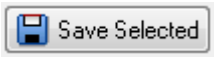
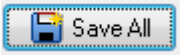
8.4.2.6 Save Data

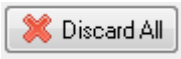
SAVE THE SESSION

Once the acquisition process ends in one box, the next window will appear.



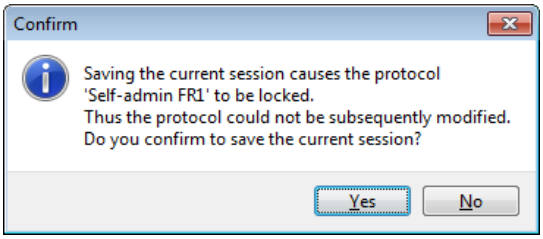




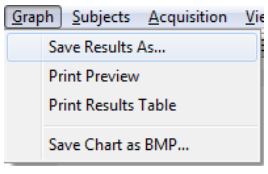


- Use the Select button to select the boxes which data will be saved.
- Use the Discard button to select the boxes which data will be not saved.
- Press the Save Selected button to save the selected boxes.
- Press the Save All button to save all the data of all the boxes (the box selection will not be taken into account).
- Press the Discard All button to discard the data of all the boxes and exiting from the saving panel.

After running a protocol for the first time in an experimental file, clicking the **Save** button will lead to the following message indicating that the associated protocol will appear as locked in the Protocols organizer table.



SAVE THE DATA TABLES



To save the raw data tables, select **Save results As…** from the **Graph** menu.

The system will show the **Report Sheets Selector** dialog, where you can select which data tables you want to export.

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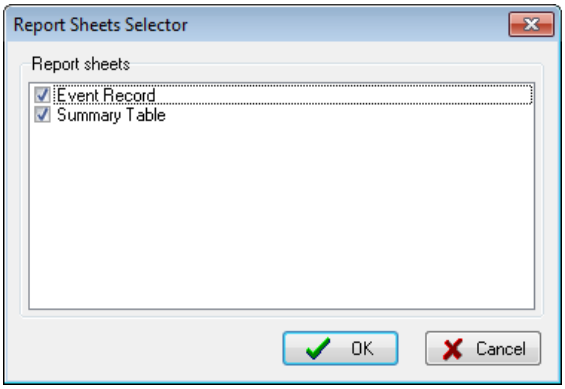
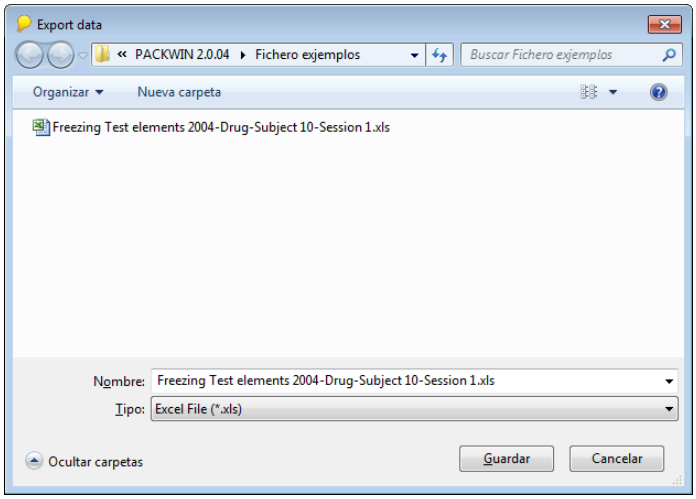


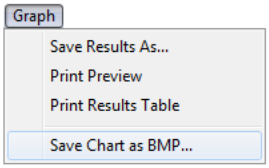
Select the reports that will be generated and exported and press the **Ok** button. The system will show the **Export data** dialog, where you can choose the location, the file name and the type of exportation.

Enter the name of the file and press the **Save** button.



Result tables data are stored in Excel (XLS) format. The different reports will be saved into different sheets on the same workbook.

SAVE AN IMAGE OF THE ACTIVITY SIGNAL



To save the image of the analogic signal of the animal activity, select **Save Charts As BMP...** from the **Graph** menu.

The system will show the **Export data** dialog, where you can choose the location, and the file name. Press the **Save** button when done.

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8.5 Starting and stopping acquisition with the remote control

Please remember that the data acquisition process can only be started if a valid protection key is plugged in or if the trial period has not expired yet.

The use of this device can be different depending on the experimental modules purchased with the system (operant, freezing/startle).

8.5.1 PACKWIN Operant Module

8.5.1.1 Start data acquisition

In order to start independent data acquisition using the teleswitch, first start the session of the selected box by pressing the "Run box" button. Then accept the command dialog "Put the subject…" by pressing the "Start DAQ" button on the remote control.

If you want to start data acquisition consecutively in several boxes, select the first box to start, press the "Run box" button, and then press the "Start DAQ and select next" button on the remote control, instead of the "Start DAQ" button. In this way, the remote control will start the selected box and automatically activate the "Run box" button of the next box, etc….This way you can continue putting subjects in each box and starting each session without having to press the "Run box" button individually for each box from the computer.

To start data acquisition of several boxes simultaneously using the teleswitch, press button "Run all boxes". Then, in dialog "Select Boxes to Run", click in button "Execute All Boxes" to start data acquisition of all boxes, or select at least one of the boxes in the list and click in button "Execute Selected Boxes" to start data acquisition but only of the selected boxes. Finally, accept the common dialog "Subjects to Run" (Put every animal in its own box…) by pressing the "Start DAQ" button on the remote control.

8.5.1.2 Stop Data Acquisition

In order to stop all the running sessions, you have to press the "Stop DAQ" button twice (at a very short interval time). This will stop all the running sessions and the command dialog "Save data" will be shown. The boxes cannot be stopped individually with the remote-control device.

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8.5.2 PACKWIN Freezing and Startle Module (CSFR and CSST)

8.5.2.1 Start data acquisition

In order to start a data acquisition through the teleswitch, first start the acquisition by selecting the Acquisition->Start Acquisition menu option. Then you can accept the command dialog "Put every animal ..." by pressing the "Start DAQ" button on the remote control.

8.5.2.2 Stop Data Acquisition

In order to stop a running session, you have to press the "Stop DAQ" button twice (at a very short interval time). This will stop the running session and the command dialog "Save data" will be shown.

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9 Data Analysis

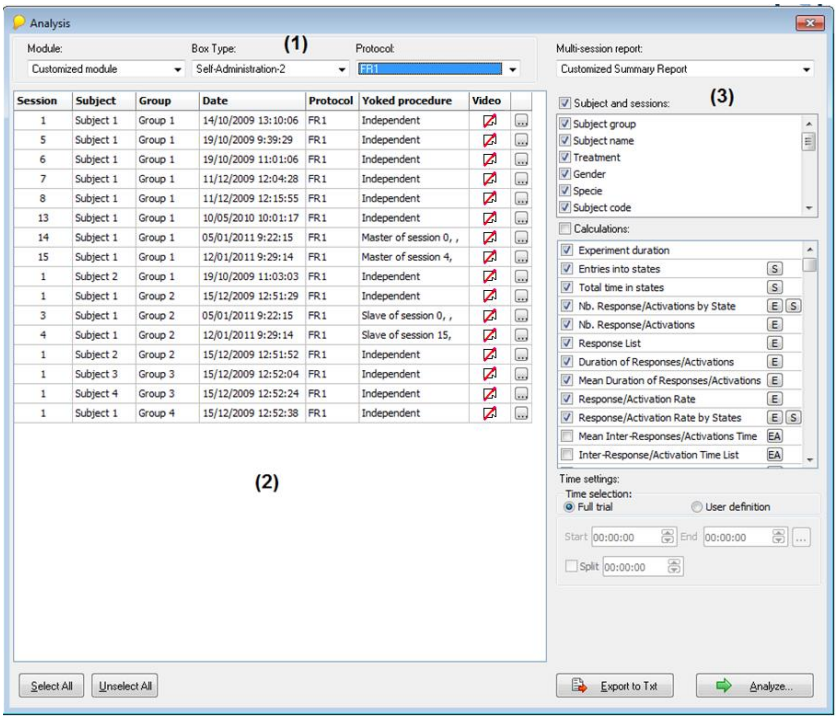
PACKWIN provides a powerful, flexible, easy-to-use analysis module for generating data directly related to the running experiments.

The analysis of the registered sessions can be performed one by one ("single session analysis report") or simultaneously through batch analysis ("Multi-session report" analysis).

9.1 The Analysis main window

The analysis main window is divided in 3 principal sections:

Session Filter (1)
Session summary table (2)
Analysis configuration panel (3)



9.1.1 Session Summary Table

The Session Summary Table shows the list of the sessions that match the filter criteria, one session for each row. The table header contains the following columns:

- Session: Session number for the subject.
- Subject: ID of the Subject which was utilized in the session.
- Group: Group's ID to which the subject belongs.
- Date: Date on which the session was registered.
- Protocol: Name of the protocol that was executed during the session.

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- Duration: Duration of the session, in [hh:mm:ss] format.
- Yoked procedure: The yoked role of the subject during the acquisition
    - Independent Box.
    - Master Box.
    - Slave of a Master Box.
- Video: The Video Recording Info of the session:
    - No Video Recording Info,

      For example, for older sessions or sessions that were not assigned a camera during acquisition.
    - Video Recording Info is broken.

      This may happen in 3 cases

        - The video file has been renamed: in this case, it is necessary to rename again the video using the name that appears in the video information window.
        - The video has been moved: in this case, the video shall be placed again in the path it was originally.
        - The video has been deleted: in this case, unless the user is able to recover the file with some kind of data recovery procedure, it will not be able to restore it.

      Refresh the Analysis window to update the status of the icons.
    - Video Recording Info is OK.
- [...]: Single-Session Analysis Report button.

9.1.2 Session Info

The Session Info section provides extra information about the selected session.

When the selected session has Video Recording Info, the details of the Video Recording Info will be shown as plain text:

- Video Camera Name.
- Video File Name.
- Video RecordingID.
- Video UTC Start Time.
- Video Config File Name.
- Video Sync. Delay (ms).

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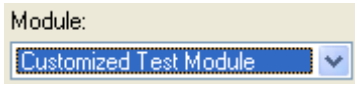
9.2 Filter the sessions to analyze

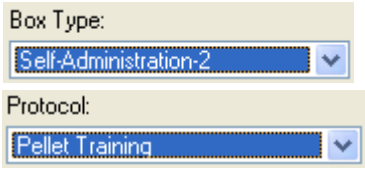
The sessions are filtered by 3 different but related items: Module, Box Type, and Protocols.



Select a module from the "Module" drop down list. After this, the application will search for box types that are compatible with the module you selected.

Depending on the modules licensed and the system used, the following analysis modules are available:

- For OPERANT modules:
  - The **Customized** module (PACKWIN-CS) provides a set of reports entirely customizable.
  - The **Nine Hole Test** module (PACKWIN-HO) provides a predefined report related to standard 5-CSRT task experiments.
  - The **Vogel Test** module provides (PACKWIN-VT) a predefined report related to standard Vogel Test experiments.
- For STARTLE/FREEZING experiments, a single option will be provided in the Module list depending on the kind of experiment opened:
  - The **Startle module** (PACKWIN-CSST) provides a predefined report related to Startle Reflex experiments.
  - The **Freezing module** (PACKWIN-CSFR) provides a predefined report related to Fear Conditioning experiments.



Select the box type from the "Box Type" drop down list. The application will list the sessions available associated with the selected box type.

Select the protocol from which sessions need to be analysed from the "Protocols" drop down list. The sessions registered with the selected protocol will be displayed in the Session summary table. It is not possible to analyse simultaneously sessions registered with different protocols.

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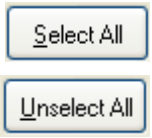
9.3 Select the sessions to analyze

The session summary table displays the sessions (1 by row) with related information. Selected sessions are highlighted with a clear blue colour.



Use the **Select All** button to select all the sessions of the table.

Use the **Unselect All** button to unselect all the sessions of the table.

Use the left button of the mouse to **select several consecutive rows**.

Session	Subject	Group	Date	Protocol	Yoked procedure	
1	Subject 1	Control	12/04/2010 17:14:35	Self-adm Prog	Independent	...
2	Subject 1	Control	13/04/2010 11:58:03	Self-adm Prog	Independent	...
1	Subject 2	Control	12/04/2010 17:43:46	Self-adm Prog	Independent	...
1	Subject 6	Control	12/04/2010 17:46:23	Self-adm Prog	Independent	...
1	Subject 3	Drug	12/04/2010 17:43:44	Self-adm Prog	Independent	...
1	Subject 4	Drug	12/04/2010 17:46:23	Self-adm Prog	Independent	...

Use the combination of the Mouse-left button and the CTRL keyboard key to **select separated rows**.

Session	Subject	Group	Date	Protocol	Yoked procedure	
1	Subject 1	Control	12/04/2010 17:14:35	Self-adm Prog	Independent	...
2	Subject 1	Control	13/04/2010 11:58:03	Self-adm Prog	Independent	...
1	Subject 2	Control	12/04/2010 17:43:46	Self-adm Prog	Independent	...
1	Subject 6	Control	12/04/2010 17:46:23	Self-adm Prog	Independent	...
1	Subject 3	Drug	12/04/2010 17:43:44	Self-adm Prog	Independent	...
1	Subject 4	Drug	12/04/2010 17:46:23	Self-adm Prog	Independent	...

To facilitate the session selection, the data can be organized by alphabetic or chronological order referring to a selected column (Session, Subject, Group, Date, Protocol, Duration...).

The "Session Info" panel shows how many sessions have been selected.

Otherwise, the Remarks field of the selected session is shown if any.

The registered sessions can be analysed by using the single session analysis tool (individual analysis of each session, one by one) or the batch analysis tool (simultaneous analysis of a batch of sessions).

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9.4 Generate a single-session analysis report

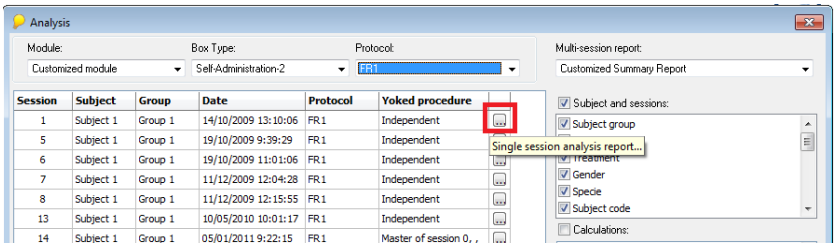
Press the **Single Session Analysis report button...** located in the last column of each session loaded into the session table.

PACKWIN allows the individual analysis of each session one by one. Different single-session analysis report is provided depending on PACKWIN modules used.

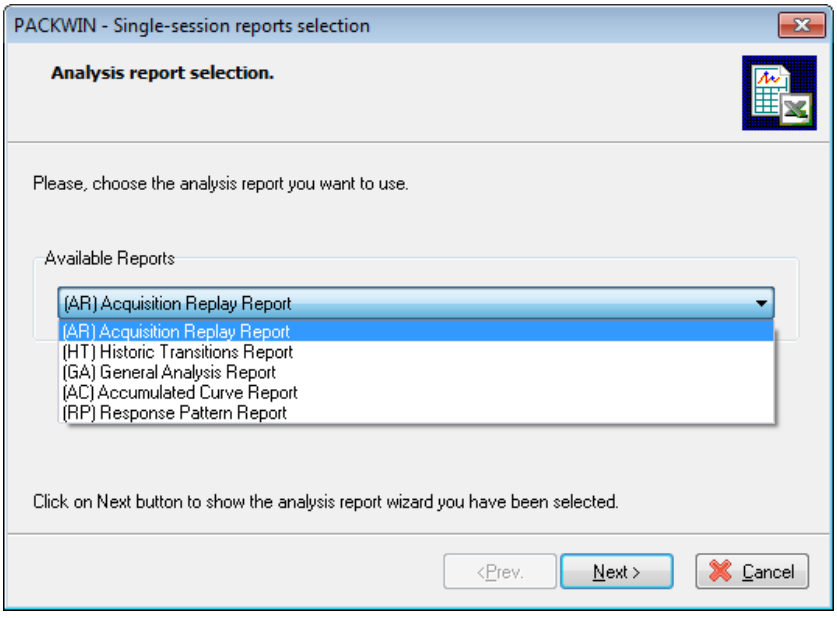
9.4.1 PACKWIN Operant Modules (CS, HO and VT)

PACKWIN-CS module provides a single session analysis area in which 5 data reports can be generated individually for each session.

Press the **Single Session Analysis report button...** located in the last column of the session table.



If an Operant Conditioning system is active, select one of the five available reports from the **Single-session reports selection** panel and press the **Next** button.



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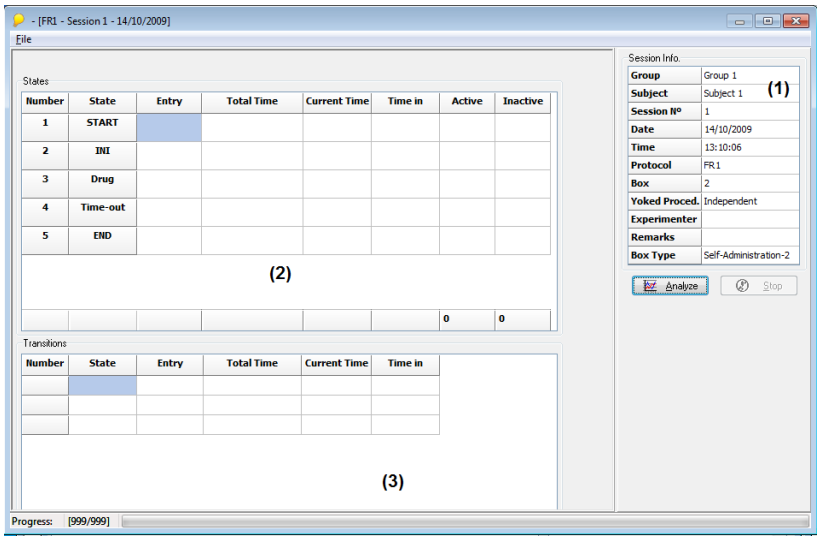
- **Acquisition Replay Report**: replays the state and transitions tables generated during the acquisition process.
- **Historic Transitions Report**: provides a chronological list of all the events (light ON, lever pressed, pellet given…) which have occurred during the session.
- **General Analysis Report**: allows obtaining a great number of additional calculations on a specific response.
- **Accumulated Curve Report**: generates cumulative response curves to depict the animal performance during the experiment.
- **Response Pattern Report**: allows to chart the events as a function of time.

9.4.1.1 Acquisition Replay Report. Operant Conditioning systems

The **Acquisition Replay Report** allows replaying the state and transitions tables generated during the acquisition process. This report gives the possibility to generate a new set of states and transitions tables from different parameters than those calculated during the original acquisition.

THE ANALYSIS WINDOW

The Analysis window shows data about the session **(1)** and displays the States **(2)** and Transitions tables **(3)** as previously configured by the user in the **Run-Time panel** section (see chapter 7.2.7.).



The position as well the parameters reported in the tables can be modified by previously entering the modifications in the **Data Configuration** module (see chapter 3.4.4).

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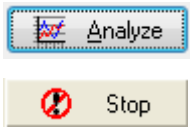
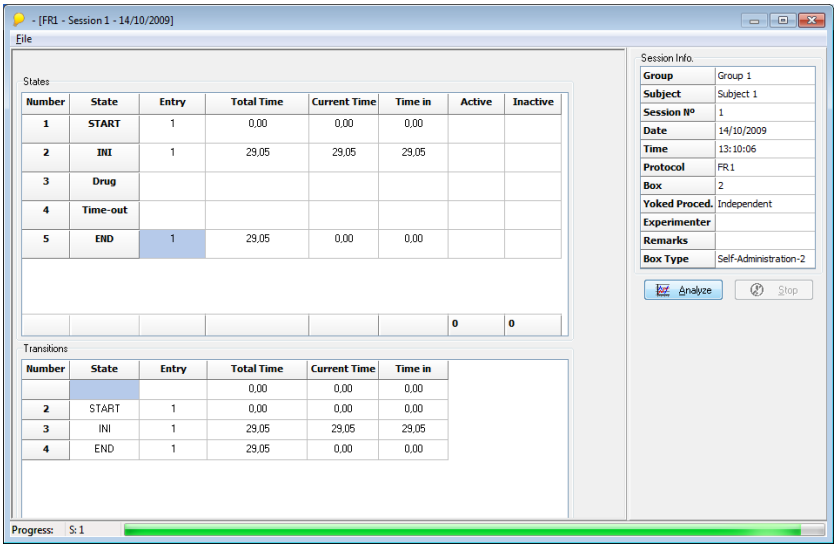
GENERATE THE DATA

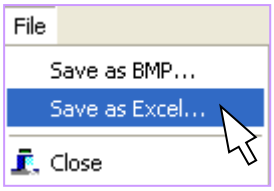


Run the Analysis process by pressing the **Analysis** button.

The Analysis process can be interrupted by pressing the **Stop** button.

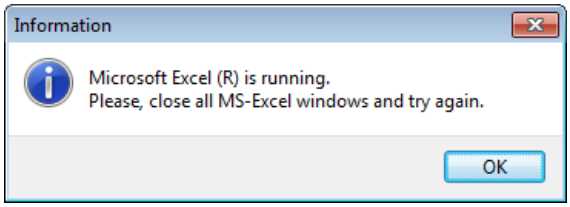


SAVE THE ANALYSIS RESULTS TO EXCEL

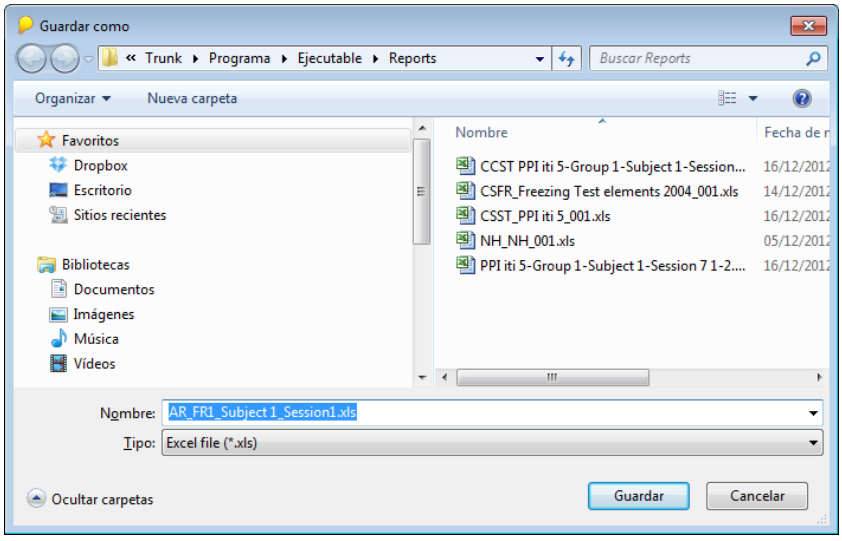


Data related to the analysis can be saved in an Excel file: Select **Save as Excel...** from the **File** menu.

The exportation will not be allowed if an excel file is opened.



Select a folder where the data will be sent, change the name of the file if desired (a name with the "AR" prefix is entered by default), press the save button.



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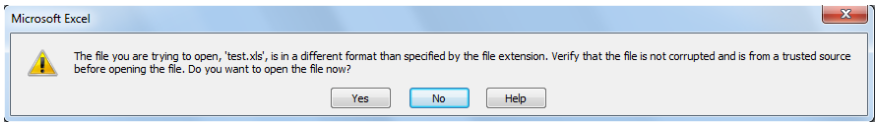
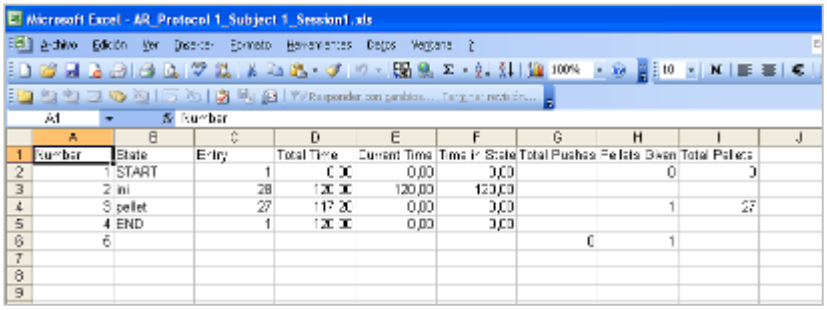
When the exported '.xls' file is opened with Excel 2010 or higher the following message may be shown depending on the version of Excel and the updates installed:



Please ignore this message and click on the 'Yes' button to open the document.

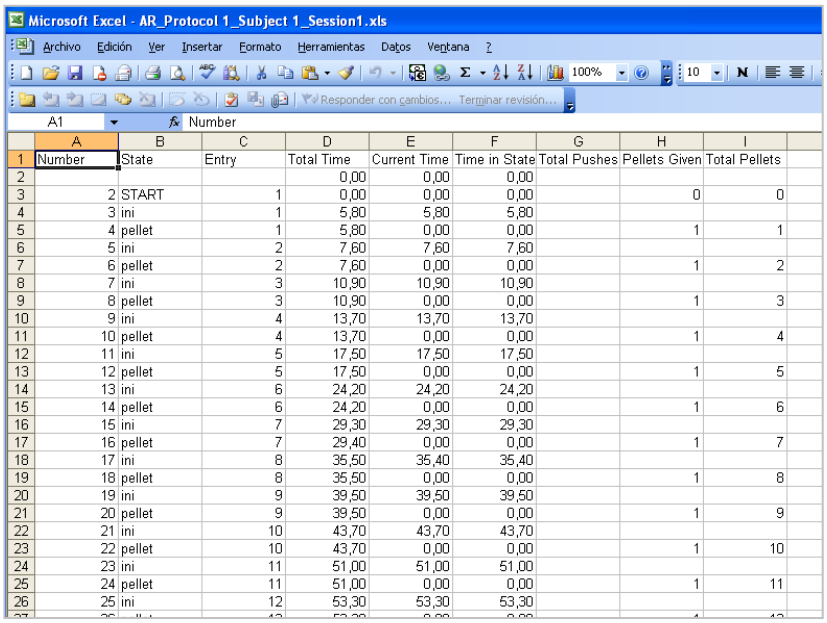
The generated Excel file reports the general information about the session, the State and Transitions table in separated Excel sheets.

Example of **State** table:



Number	State	Entry	Total Time	Current Time	Time in State	Total Pushes	Pellets Given	Total Pellets
1	START	1	0,00	0,00	0,00		0	0
2	ini	28	120,00	120,00	120,00			
3	pellet	27	0,00	0,00	0,00		1	27
4	END	1	0,00	0,00	0,00			
6						0	1	

Example of **Transition** table:



Number	State	Entry	Total Time	Current Time	Time in State	Total Pushes	Pellets Given	Total Pellets
			0,00	0,00	0,00			
2	START	1	0,00	0,00	0,00		0	0
3	ini	1	5,80	5,80	5,80			
4	pellet	1	5,80	0,00	0,00		1	1
5	ini	2	7,60	7,60	7,60			
6	pellet	2	7,60	0,00	0,00		1	2
7	ini	3	10,90	10,90	10,90			
8	pellet	3	10,90	0,00	0,00		1	3
9	ini	4	13,70	13,70	13,70			
10	pellet	4	13,70	0,00	0,00		1	4
11	ini	5	17,50	17,50	17,50			
12	pellet	5	17,50	0,00	0,00		1	5
13	ini	6	24,20	24,20	24,20			
14	pellet	6	24,20	0,00	0,00		1	6
15	ini	7	29,30	29,30	29,30			
16	pellet	7	29,40	0,00	0,00		1	7
17	ini	8	35,50	35,40	35,40			
18	pellet	8	35,50	0,00	0,00		1	8
19	ini	9	39,50	39,50	39,50			
20	pellet	9	39,50	0,00	0,00		1	9
21	ini	10	43,70	43,70	43,70			
22	pellet	10	43,70	0,00	0,00		1	10
23	ini	11	51,00	51,00	51,00			
24	pellet	11	51,00	0,00	0,00		1	11
25	ini	12	53,30	53,30	53,30			

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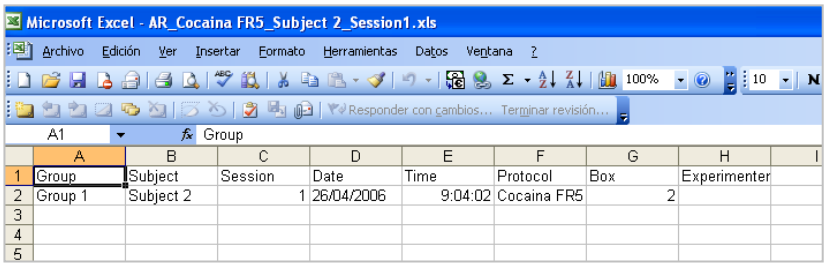
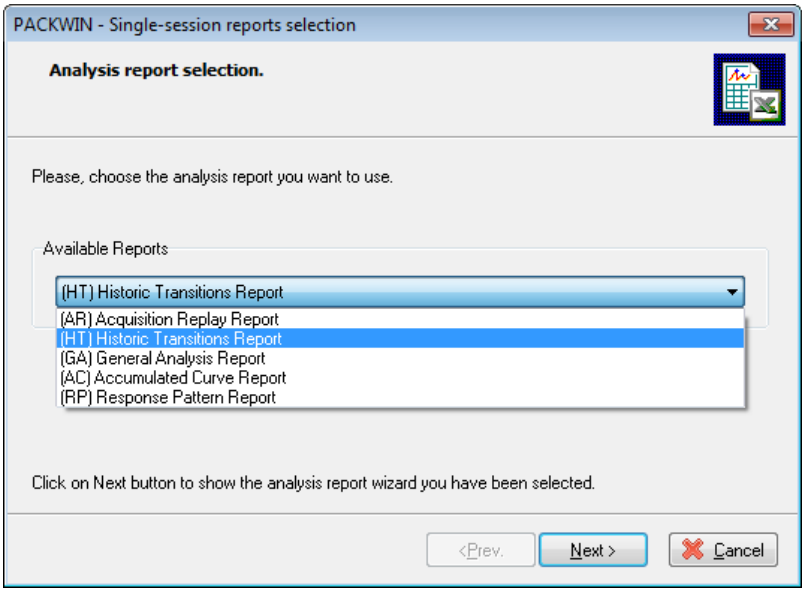
Example of **Summary** table:



	A	B	C	D	E	F	G	H
1	Group	Subject	Session	Date	Time	Protocol	Box	Experimenter
2	Group 1	Subject 2	1	26/04/2006	9:04:02	Cocaina FR5	2	
3								
4								
5								

9.4.1.2 Historic Transitions Report

The **Historic Transitions Report** provides a chronological list of all the events (light ON, lever pressed, pellet given…) which have occurred during the session. It can be used as a technical report for checking whether the protocol has been executed correctly during the session.



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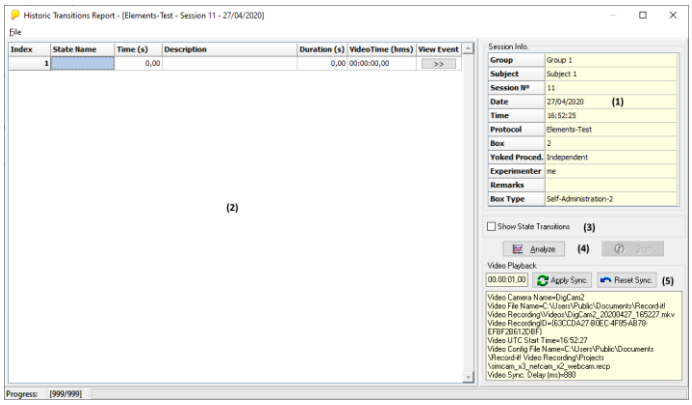
THE ANALYSIS WINDOW

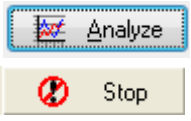
The Analysis window shows data about the session info **(1)**, a table **(2)**, the "Show State Transitions" options **(3)**, the report options **(4)**, and the video recording info **(5)**.



The Historic Transitions Report has the following columns:

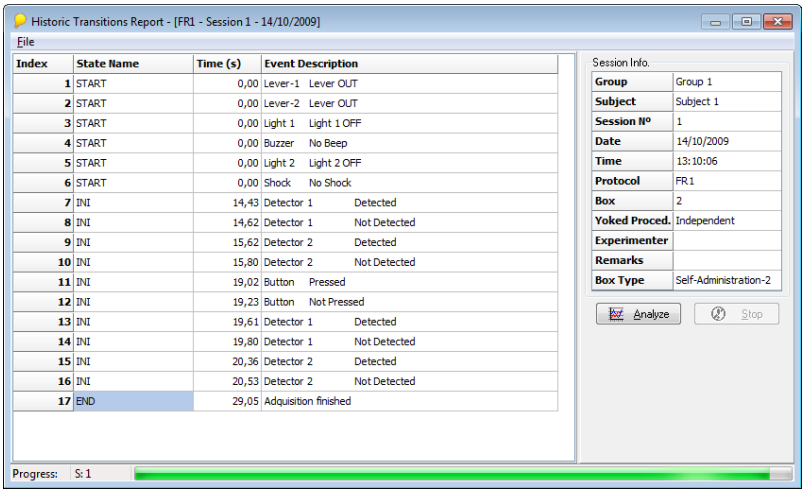
- Index: Incremental counter.
- State Name: Protocol's State when the event occurs.
- Time (s): Event time in seconds.
- Description: Event description.
- Duration (s): Event duration in seconds.

GENERATE THE DATA



Run the Analysis process by pressing the **Analyse** button.

The Analysis process can be interrupted by pressing the **Stop** button.



Mark the "Show State Transitions" in order to obtain the protocol state transitions throughout the execution of the protocol interspersed with the session events.

SYNCHRONIZED VIDEO

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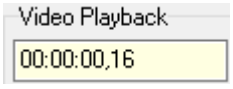
The Video Recording Info section is shown when the synchronized video recording feature had been used during the data acquisition. This feature adds two new columns to the report:

- **VideoTime:** video position in [hh:mm:ss.dd] format where the event occurred.
- **View:** seek tool to playback a video-clip from the **VideoTime** position during the **Duration** time.

The first time the **View** button [>>] is pressed, the Record-it! Media application is launched, and the related video file is loaded into the integrated player, the player jumps to the indicated position, and plays the video for the duration of the recording.

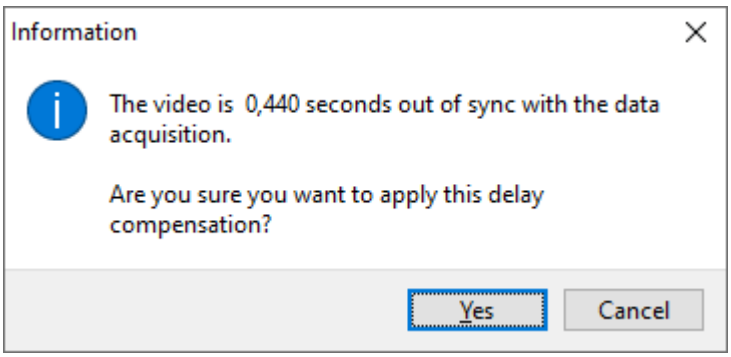


During the video-clip playback, the field [Video Position] is updated.

Sometimes there is a small delay between the recorded event and the image on the video. To make a fine adjustment between the time of the event and the position of the video, you can use the "Apply Sync" tool, following the steps below:

1) Locate in the video in which position a certain event starts, using the navigation and playback buttons integrated in the Player. For example, at position 00:01:23.44.
2) Locate the event in question in the transition table and select the row of this event by clicking on one of the cells of the row. For example, at VideoTime 01:23.00.

3) Press the "Apply Sync" button. The program will recalculate the "VideoTime" column to apply the found offset.
4) Confirm the offset by pressing **Yes** in the confirmation dialog:



In order to reset the offset to zero ms to start over, you can press the Reset Sync button. This will re-calculate the "Video Time" column applying an offset of 0 seconds.

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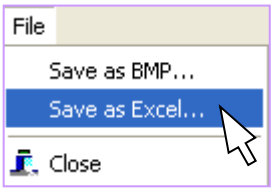
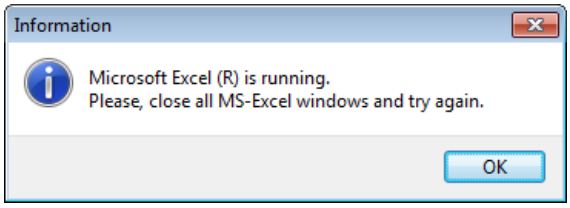
SAVE THE ANALYSIS DATA

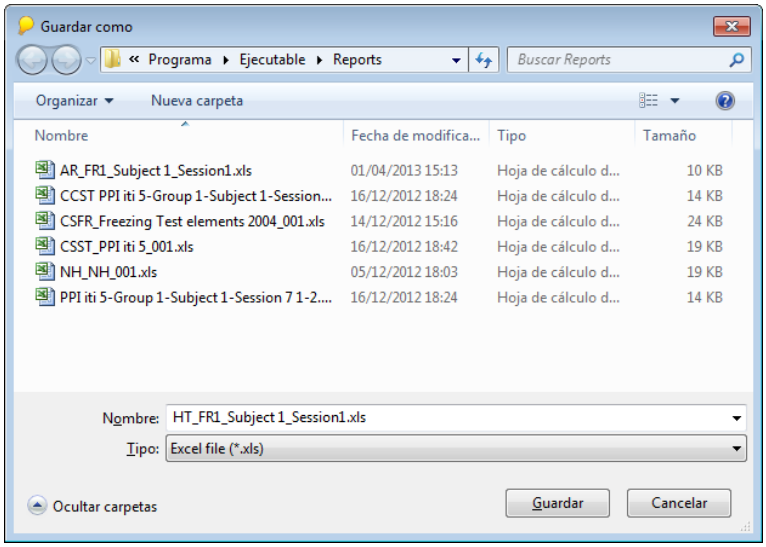


Analysis results can be saved in an Excel file. Select **Save as Excel...** from the **File** menu.

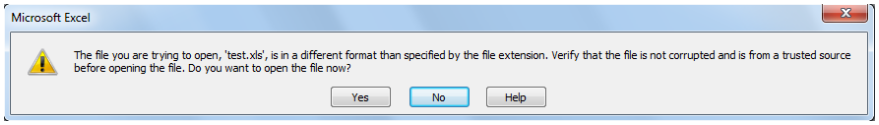
The exportation will not be allowed if an Excel file is opened.



Select a folder where the data will be sent, change the name of the file if desired (a name with the "HT" prefix is entered by default), press **save.**



When the exported '.xls' file is opened with Excel 2010 or higher the following message may be shown depending on the version of Excel and the updates installed:



Please ignore this message and click on the 'Yes' button to open the document.

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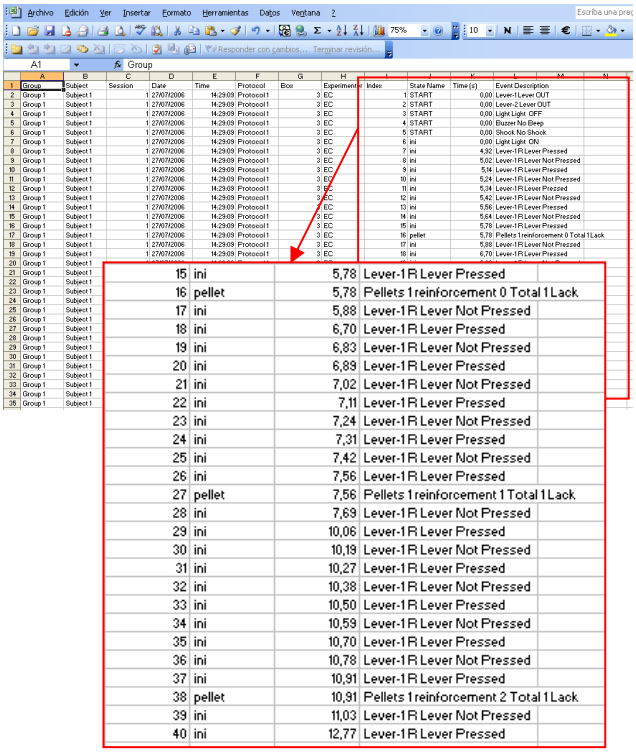
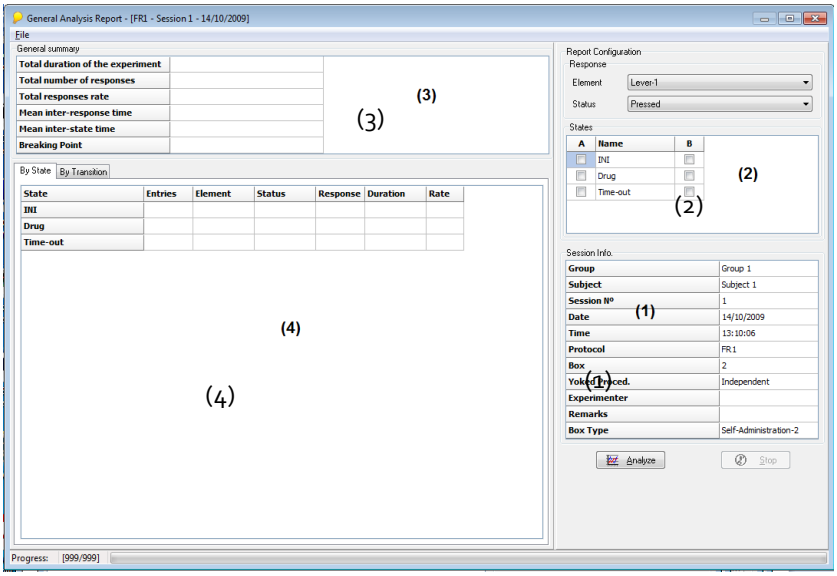
The Excel file reports the general information about the session and summarizes the data of the Historic report for further analysis.



| | | |

THE ANALYSIS WINDOW

The Analysis window shows data about the session **(1)**, a configuration panel **(2)**, a general summary panel **(3)**, and two tables of results **(4)**.



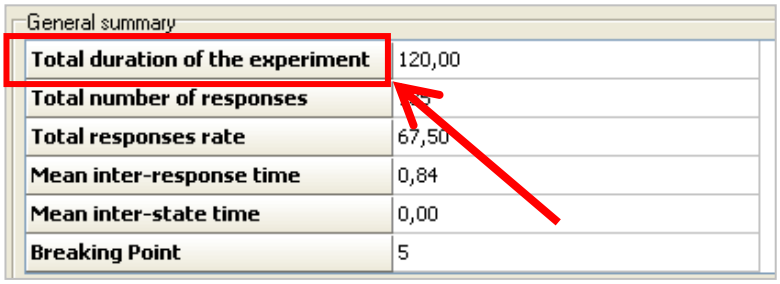
The General Report contains three kind of data:

- independent data (given automatically without any configuration),
- response-dependent data (dependent on the responses selected by the user) and (iii),
- state-dependent data (depends on the states selected by the users).

Independent data

Only two of the data provided in this report are independent of the previous selection of a response or of a state by the user:

--Total duration of the experiment, given in seconds (see General summary table).



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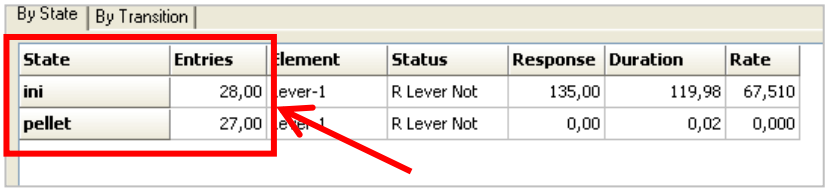
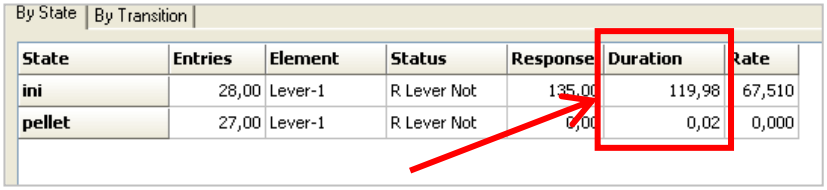
- Number of **Entries** in each states of the protocol (see **By State** table).



- the **Total Duration of each state** in the experiment, given in seconds (see **By State** table).

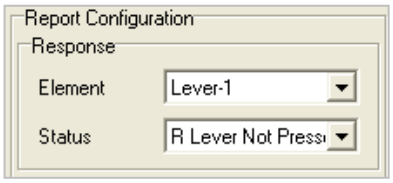


Response-dependent data

Selection of the response under study:

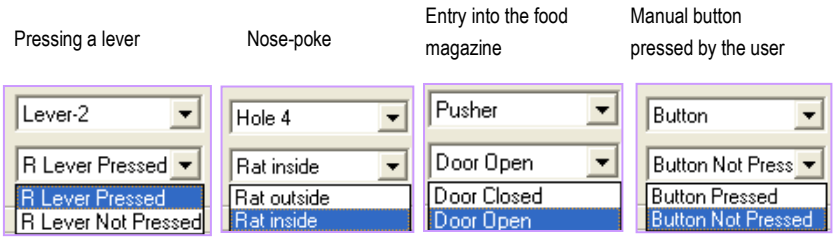
The report provides some calculations related to a response selected by the user in the configuration panel.

Choose the **Element** from the list (lever, nose-poke...) and the **Status** of the element to be represented.



The elements available in the list correspond to the elements used as "**Input elements**" during the acquisition of the data and will depends on the personal configuration of the customer; basically from the jaulas.box file provided with the system.

These are some example of "Input elements":



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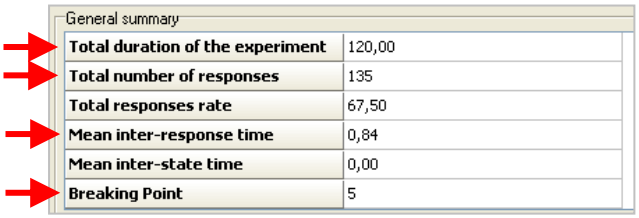
Data provided:

- ***Total number of responses*** (see General summary table).

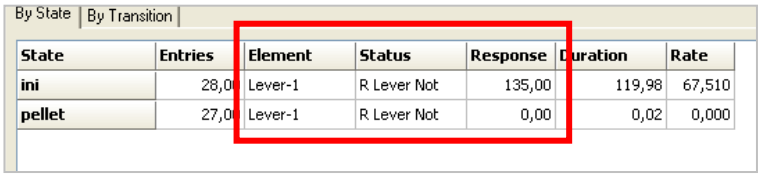
- ***Total response rate***, given in number of resp./min (see ***General summary*** table): total number of response per min considering the whole session.

- ***Mean inter-response time, given in seconds*** (see ***General summary*** table): mean of all the intervals of time between the consecutives responses.

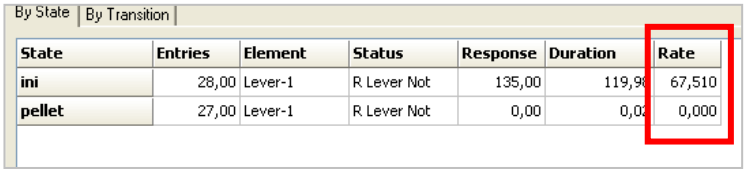
- ***Breaking Point***: number of times the subject has to perform the selected response in the current state for inducing a change to the next state.



- ***Total number of response by state*** (see ***By State*** table): the number of times the selected response (indicated in the ***Element*** and ***Status*** columns) occurs (***Response*** column).



- ***Response Rate in each state***, given in number of resp./min (see ***By State*** table): rate (***Rate*** column) of the selected response ((indicated in the ***Element*** and ***Status*** columns) in each state.



- ***Inter-response time*** (***I.R.T.***), expressed in seconds (see ***By Transition*** table): list of all the intervals of time between two consecutive selected responses.

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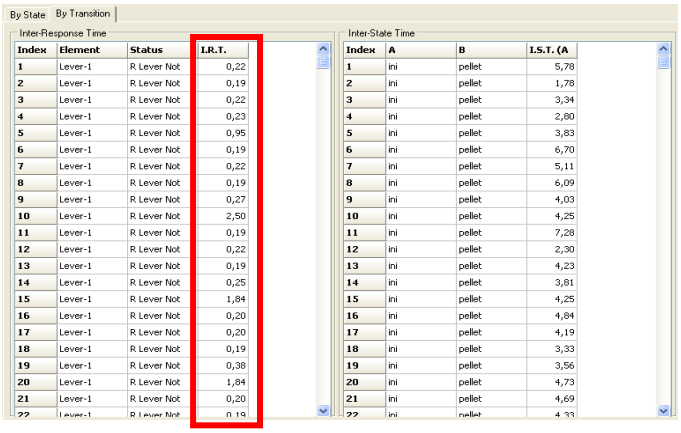
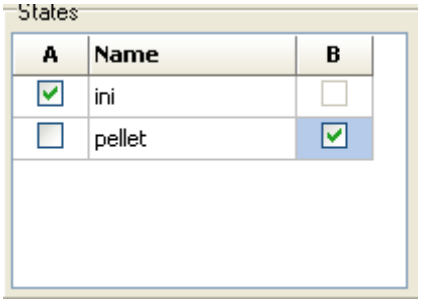


State-dependent data

Basically, the General Report gives calculation of intervals of time between two different states selected by the user in the configuration panel.

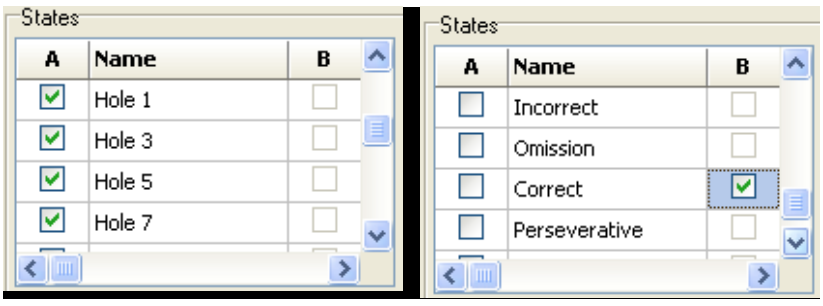
Selection of the states under study:

Choose the two states (***A***: start state and ***B***: end state for the calculation) to be considered for the calculation by checking the corresponding check box. In the next example, the user wants to know with which latency the subject receives a pellet.



The states available in the list (***Name***) correspond to the states edited by the user in the protocol used during the acquisition of the data.

Several ***A*** states (but only one ***B***) can be chosen for obtaining data of interest, for example in a protocol using a 5-9 holes box.



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In the example above, the user wants to calculate the latency in performing a correct response. In his protocol this data corresponds to the interval of time between the states in which the light is switched ON in ***the Holes 1,3,5,7*** and ***9*** and the state in which the animal receives a pellet (***Correct Response***).

Data given:

- ***Mean inter-state time*** (see ***General summary*** table): mean of all the intervals of time between the user-selected states.

General summary

Total duration of the experiment	1199,13
Total number of responses	114
Total responses rate	5,70
Mean inter-response time	10,59
Mean inter-state time	0,95
Breaking Point	1

- ***Inter-state time*** (***I.S.T.***), given in seconds (**see By Transition** table): list of all the intervals of time between the selected states. Here, 2 example:

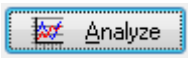
Inter-State Time

Index	A	B	I.S.T. (A
1	ini	pellet	5,78
2	ini	pellet	1,78
3	ini	pellet	3,34
4	ini	pellet	2,80
5	ini	pellet	3,83
6	ini	pellet	6,70
7	ini	pellet	5,11
8	ini	pellet	6,09
9	ini	pellet	4,03
10	ini	pellet	4,25
11	ini	pellet	7,28
12	ini	pellet	2,30
13	ini	pellet	4,23
14	ini	pellet	3,81
15	ini	pellet	4,25
16	ini	pellet	4,84
17	ini	pellet	4,19
18	ini	pellet	3,33
19	ini	pellet	3,56
20	ini	pellet	4,73
21	ini	pellet	4,69
22	ini	pellet	4,33

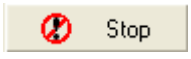
Inter-State Time

Index	A	B	I.S.T. (A
67	Hole 1	Correct	0,78
68	Hole 9	Correct	0,86
69	Hole 5	Correct	1,95
70	Hole 9	Correct	1,09
71	Hole 5	Correct	2,00
72	Hole 3	Correct	1,03
73	Hole 5	Correct	0,91
74	Hole 9	Correct	0,61
75	Hole 3	Correct	0,61
76	Hole 9	Correct	0,63
77	Hole 3	Correct	0,41
78	Hole 3	Correct	0,72
79	Hole 7	Correct	1,48
80	Hole 7	Correct	0,67
81	Hole 1	Correct	2,75
82	Hole 3	Correct	0,45
83	Hole 3	Correct	0,66
84	Hole 9	Correct	0,56
85	Hole 1	Correct	0,73
86	Hole 5	Correct	1,00
87	Hole 5	Correct	0,70

GENERATE THE DATA



Run the Analysis process by pressing the **Analysis** button.



The Analysis process can be interrupted by pressing the **Stop** button.

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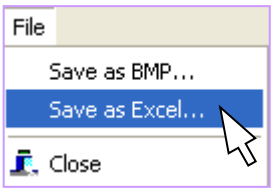
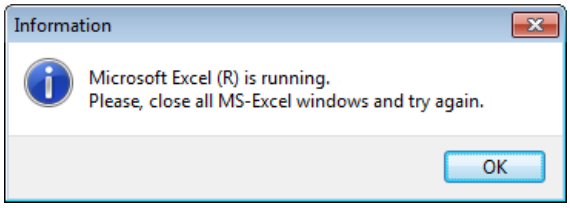


SAVE THE ANALYSIS DATA

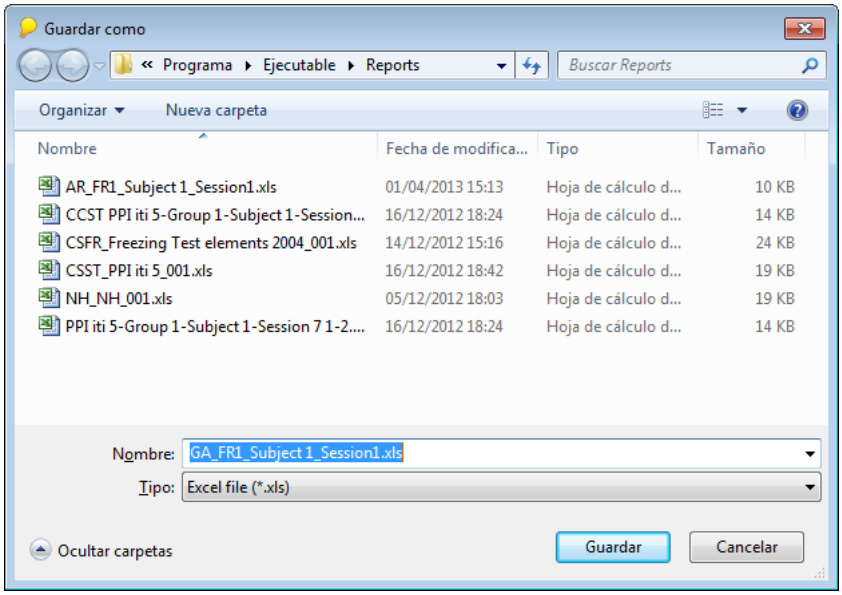
Data related to the analysis can be saved in an Excel file: Select **Save as Excel...** from the **File** menu.

The exportation will not be allowed if an excel file is opened.



Select the parts of the General report to be exported in Excel by checking the corresponding boxes.

Select a folder where the data will be sent, change the name of the file if desired (a name with the "GA" prefix is entered by default), press **save.**



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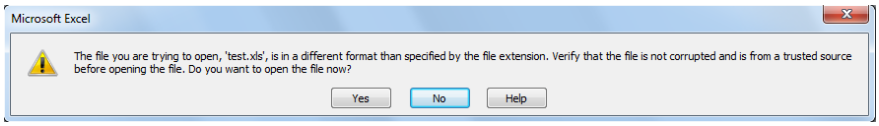
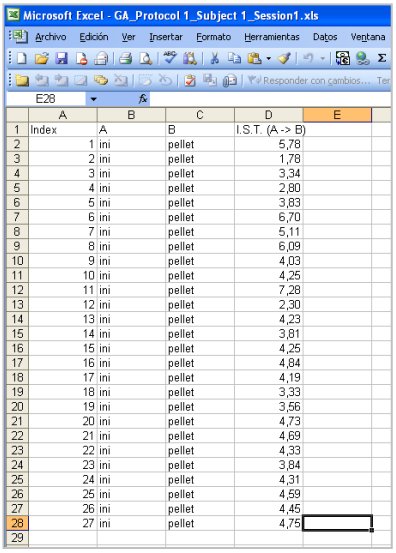
When the exported '.xls' file is opened with Excel 2010 or higher the following message may be shown depending on the version of Excel and the updates installed:

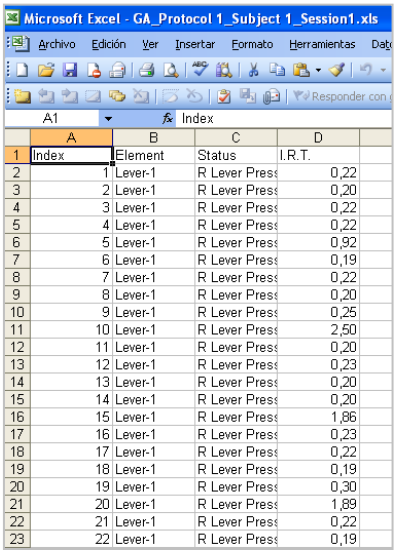


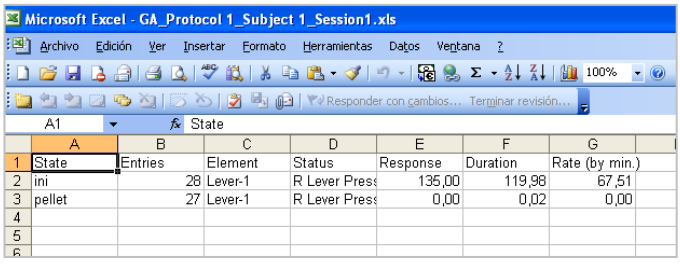
Please ignore this message and click on the 'Yes' button to open the document.

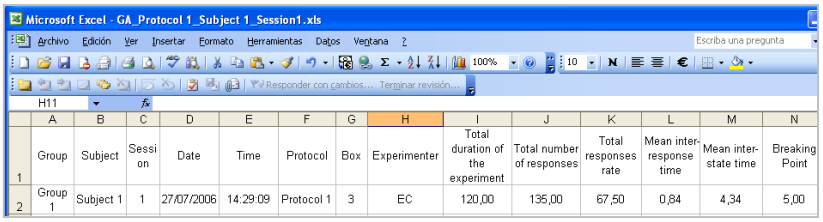
The generated Excel file reports the general information about the session and summarizes the data of the General report for further analysis. The different parts of the report are exported in different Excel sheets.

These are some examples:









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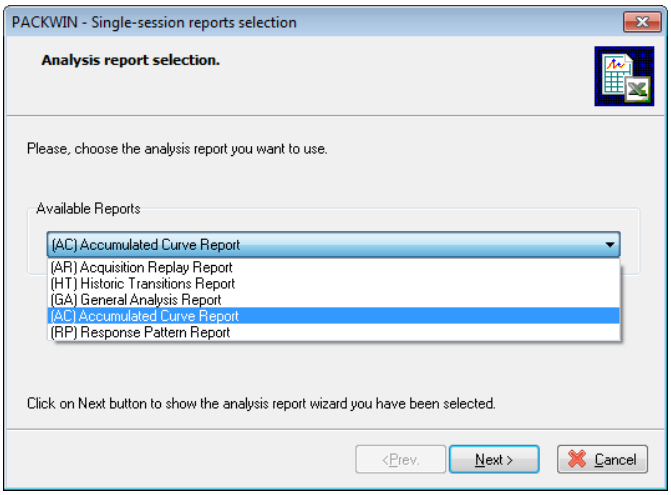
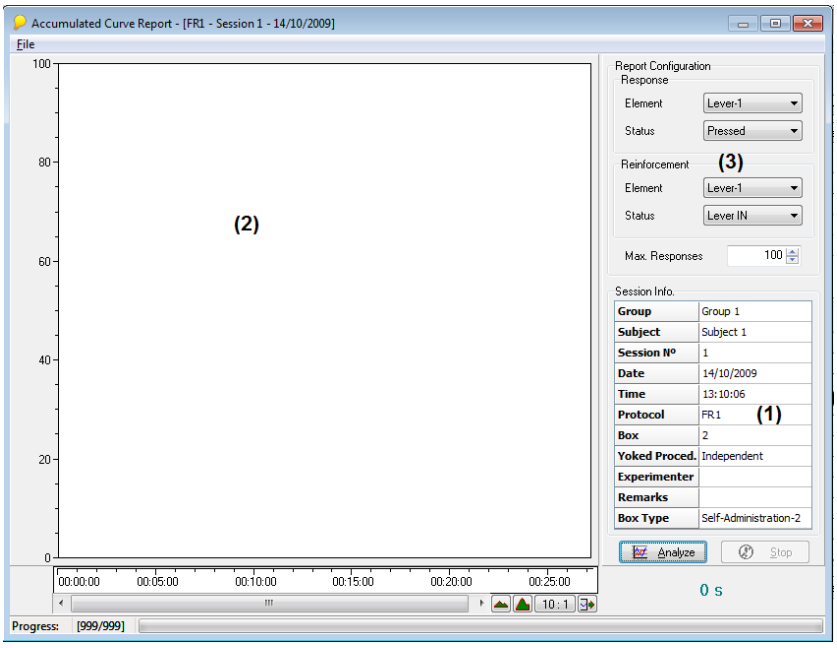
9.4.1.4 Accumulated Curve Report

The sessions recorded during the acquisition process can be opened and re-analysed for generating cumulative response curves. The cumulative response curve is used in classical operant conditioning experiments to depict the animal performance during the experiment.



THE ANALYSIS WINDOW

The Analysis window contains data about the session **(1)**, a graph area **(2)** and a parameters to set for obtaining the user-defined cumulative curve **(3)**.



©2024 Panlab

CONFIGURE THE ANALYSIS PARAMETERS

Choose the response

The response whose cumulative curve will be represented is to be selected in the **Response** section

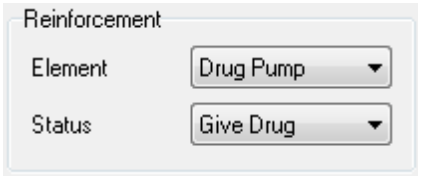
Choose the element from the list (lever, nose-poke....) and the state of the element to be represented.

The elements available in the list correspond to the elements used as "**Input elements**" during the acquisition of the data and will depends on the personal configuration of the customer; basically from the jaulas.box file provided with the system.

Choose the "reinforcement"

The cumulative curve also contains information about the reinforcement (or stimuli) received during the session.

Choose the reinforcement / stimuli to be reported in the graph in the **Reinforcement** session.



First, choose the element from the list (drug, pellet, shock, light, buzzer....) and the state of the element to be represented.

The elements available in the list correspond to the elements used as "Output **elements**" during the acquisition of the data and will depends on the personal configuration of the customer; basically from the jaulas.box file provided with the system.

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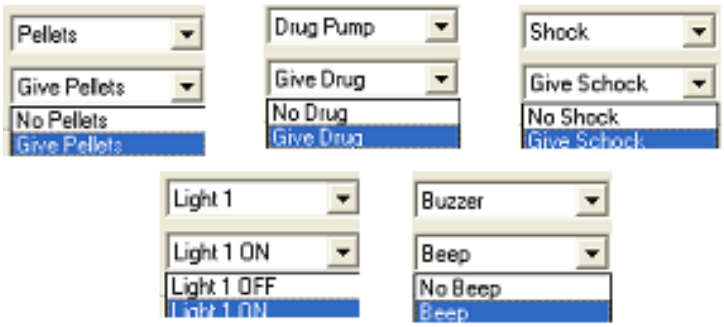
These are some examples of output elements:

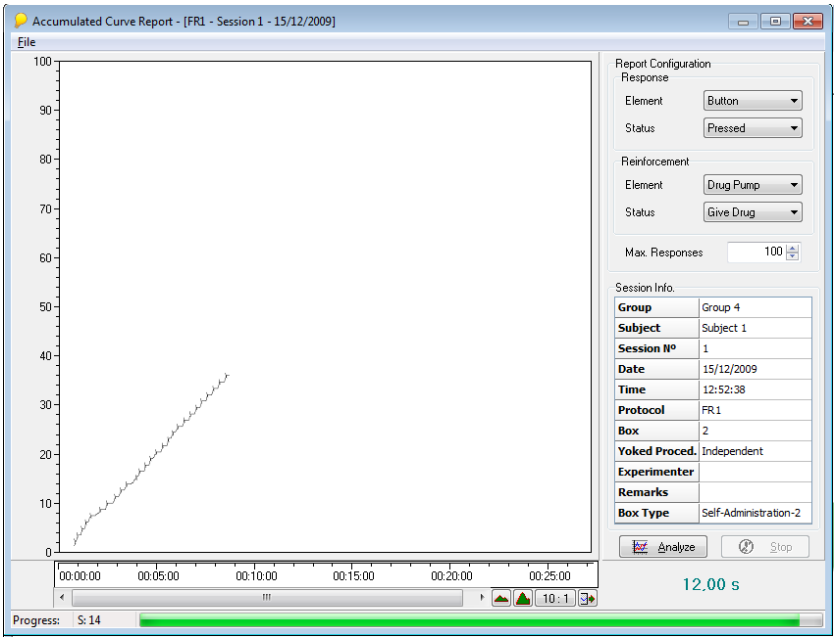


A reinforcement given at the beginning of the experiment (Time o) will not be shown in the Accumulated Curve Report

9.4.1.5 Generate the data

Once selected the parameters of the analysis, press the **Analyse** button to generate the cumulative curve.

The Analysis process can be interrupted by pressing the **Stop** button.



X axis shows the session acquisition time (hh:mm:ss). Y axis represents the cumulated number of user-defined responses.

The cumulative response is represented on black line and the user-defined reinforcement/stimuli as an oblique bar (\) on the curve.

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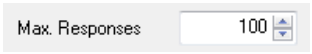
HORIZONTAL SCROLL

The horizontal scroll is used by displacing the horizontal scroll bar located just below X-time axis.

ZOOM

The X-time axis is provided of a zoom-in and zoom-out options, using respectively the and buttons located at the bottom right side of the signal. The zoom list can be also used.

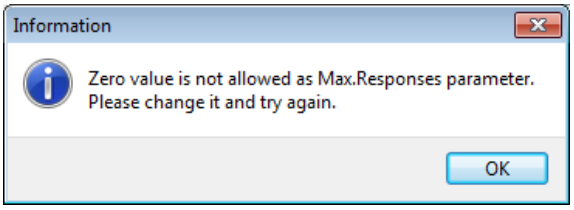
Please note that zoom is only applied in the time scale (X axis).



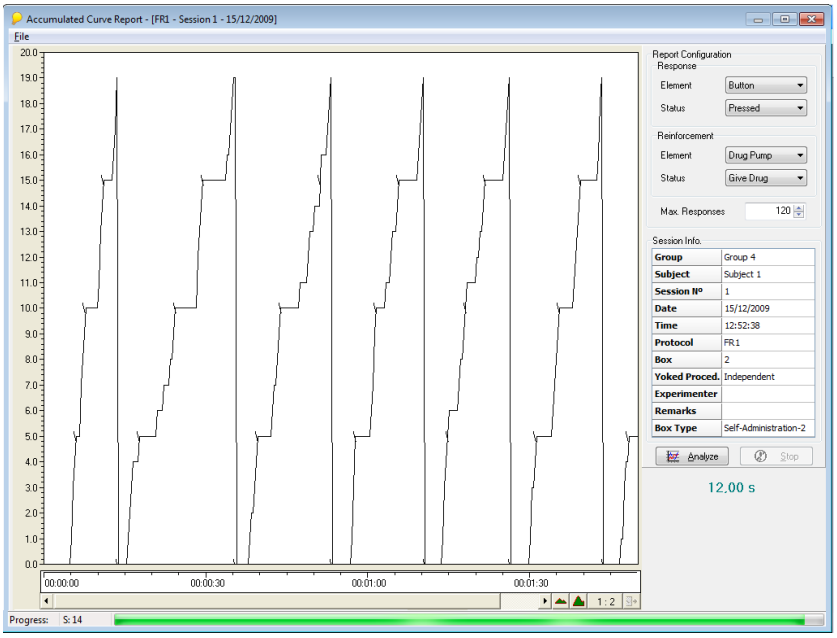
MAXIMUM RESPONSES

The top number of response for the graph can be chosen by the user. When the curve will reach this top, the curve will be reset to the bottom of the graph, causing a vertical line to be drawn down the graph from top to bottom. This option gives to the cumulative record the appearance of a mountain peaks or waves.

The **Max. Responses** value cannot be "zero".



After changing the value of the **Max.Responses**, the **Analyse** button has to be pressed again for generating the new cumulative response curve.



TOTAL DURATION OF THE SESSION

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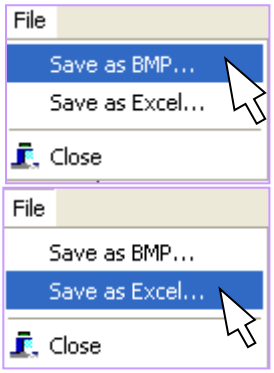
The total duration of the session is also given on the right-button part of the window.

9.4.1.6 Save the Analysis data



SAVE THE GRAPH AS IMAGE

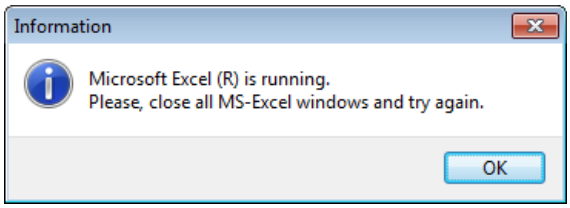
Once the reanalysis process has finished, the graph can be saved as *.bmp image file for data illustration. Select **Save as BMP...** from the **File** menu.

EXPORT THE DATA IN EXCEL

Data related to the analysis can also be saved in an Excel file: Select **Save as Excel...** from the **File** menu.

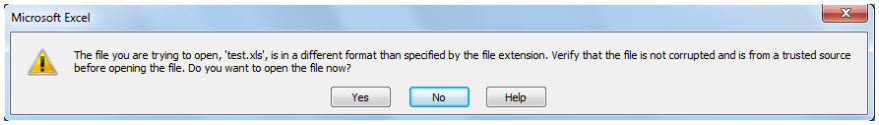
Select a folder where the data will be sent, change the name of the file if desired (a name with the "AC" prefix is entered by default), press **Save.**

The exportation will not be allowed if an Excel file is opened.



After the exportation of the data new parameters of analysis, a new analysis can be performed by choosing different analysis parameter and pressing again the **Analysis** button.

When the exported '.xls' file is opened with Excel 2010 or higher the following message may be shown depending on the version of Excel and the updates installed:



Please ignore this message and click on the 'Yes' button to open the document.

The generated Excel file reports the general information about the session and summarizes the data of the cumulative response curve (cumulative response list, and frequency of reinforcements) for further analysis.

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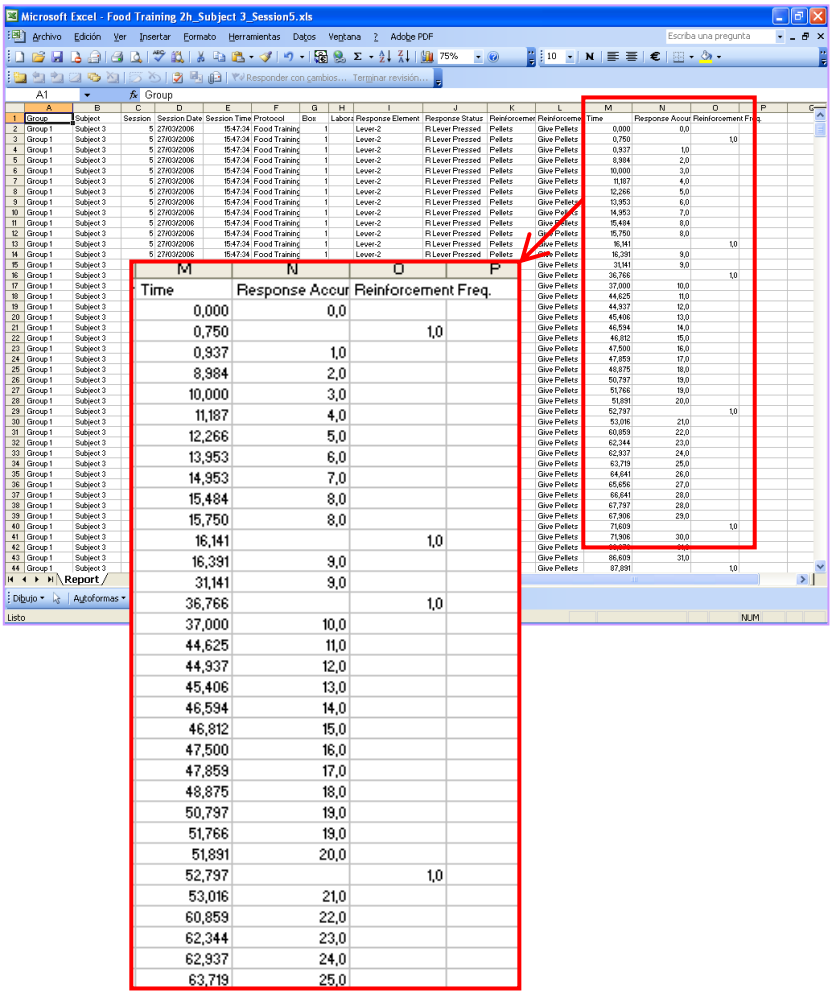
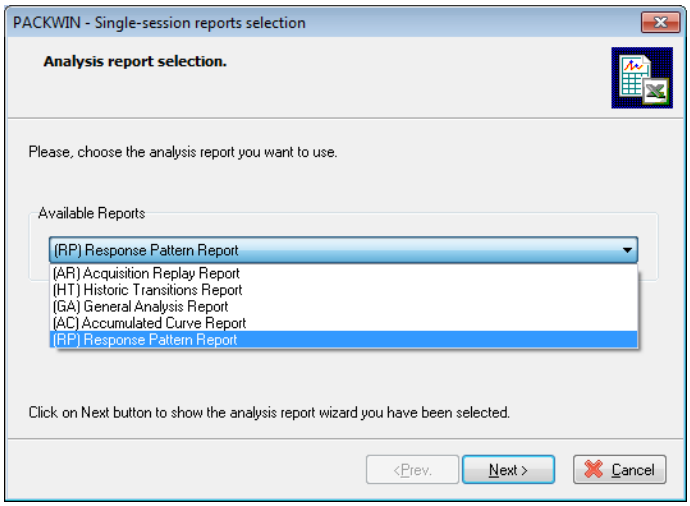


Time	Response Accur	Reinforcement Freq.
0,000	0,0	
0,750		1,0
0,937	1,0	
8,984	2,0	
10,000	3,0	
11,187	4,0	
12,266	5,0	
13,953	6,0	
14,953	7,0	
15,484	8,0	
15,750	8,0	
16,141		1,0
16,391	9,0	
31,141	9,0	
36,766		1,0
37,000	10,0	
44,625	11,0	
44,937	12,0	
45,406	13,0	
46,594	14,0	
46,812	15,0	
47,500	16,0	
47,859	17,0	
48,875	18,0	
50,797	19,0	
51,766	19,0	
51,891	20,0	
52,797		1,0
53,016	21,0	
60,859	22,0	
62,344	23,0	
62,937	24,0	
63,719	25,0	

9.4.1.7 Response Pattern Report

The **Response Pattern Report** gives a graphical representation of the temporal distribution of the user-selected responses.



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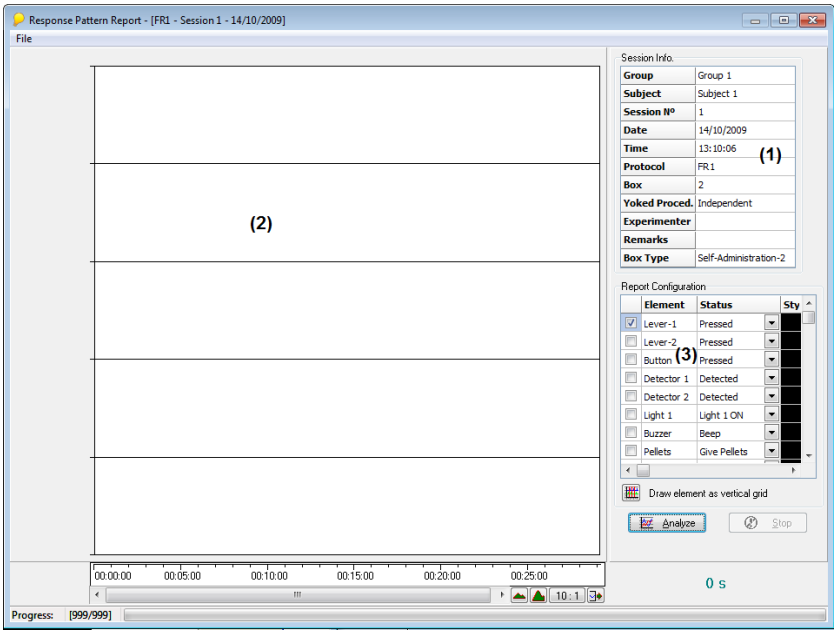
THE ANALYSIS WINDOW

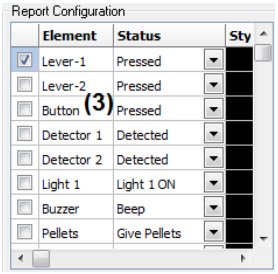
The Analysis window contains data about the session **(1)**, a graph area **(2)** and parameters to set for obtaining the user-defined cumulative curve **(3)**.



CONFIGURE THE ANALYSIS PARAMETERS

Choose the response

The responses whose pattern distribution will be represented is to be selected in the ***Response*** section. Choose the elements from the list (lever, nose-poke….) and the state of the element to be represented. A maximum of 16 patterns can be visualized at the same time.



The elements available in the list correspond to the elements used as "***Input*** elements" during the acquisition of the data and will depend on the personal configuration of the customer; basically from the jaulas.box file provided with the system.

By default, the pattern graphs are displayed in black, but a different colour can be chosen by the user by double-clicking on the corresponding case in the Style (***Sty***) column, choosing a new colour, and pressing the ***Ok*** button.

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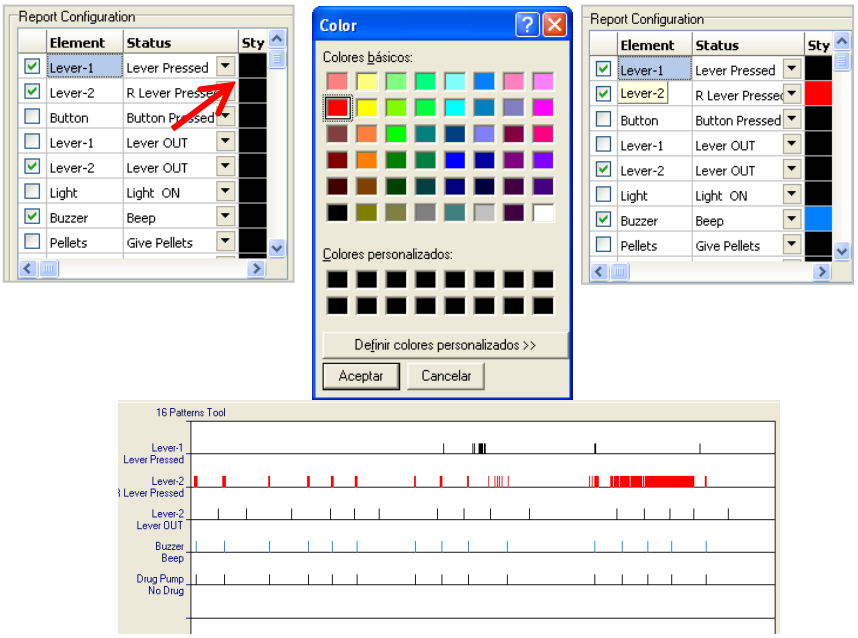
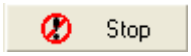


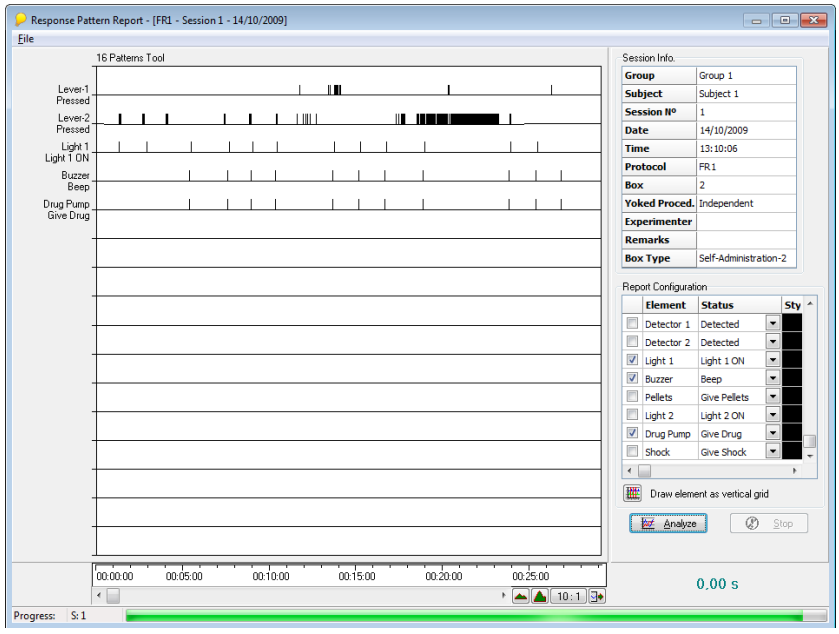
A reinforcement given at the beginning of the experiment (Time o) will not be shown in the Response Pattern Report

GENERATE THE DATA

Once you have selected the parameters of the analysis, press the ***Analysis*** button to generate the cumulative curve.



The Analysis process can be interrupted by pressing the ***Stop*** button.



X axis shows the session acquisition time (hh:mm:ss). Y axis represents the responses selected.

The occurrence of a response is represented by a black vertical line (default).

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Horizontal scroll

The horizontal scroll is used by displacing the horizontal scroll bar located just below X-time axis.

Zoom

The X-time axis is provided of a zoom-in and zoom-out options, using respectively the and buttons located at the bottom right side of the signal. The zoom list can be also used.

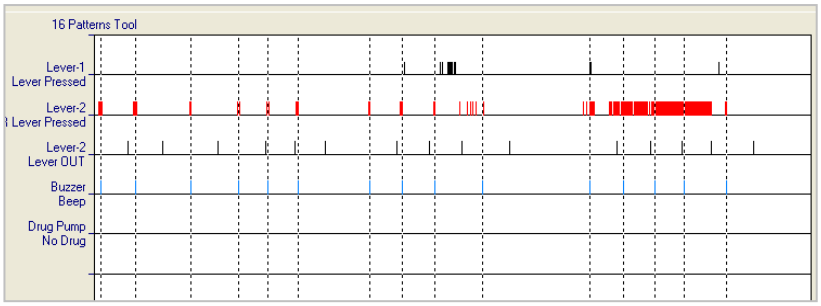
Zoom is only applied in the time scale (X axis).

Vertical reference grid

The report allows drawing vertical lines on a user-selected response (classically the reinforcement) in order to have a visual reference of when the selected response occurred with respect to the other responses.

Select a response in the **Element** column, click on the "**Draw element as vertical grid**". The name of the element will appear in bold format. ...And press again the **Analyse** button.

In the next figure, the drug administration is taken as reference.



Total duration of the session

The total duration of the session is also given on the right- bottom part of the window

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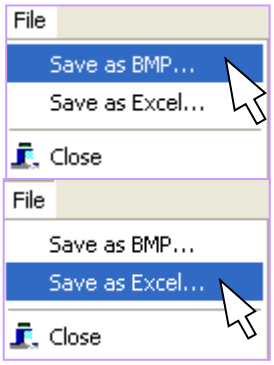
SAVE THE ANALYSIS DATA



Save the graph as image

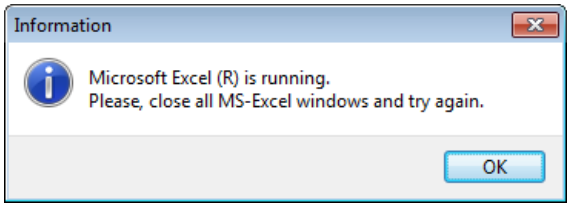
Once the reanalysis process has finished, the graph can be saved as *.bmp image file for data illustration. Select **Save as BMP...** from the **File** menu.

Export the data in Excel

Data related to the analysis can also be saved in an Excel file: Select **Save as Excel...** from the **File** menu.

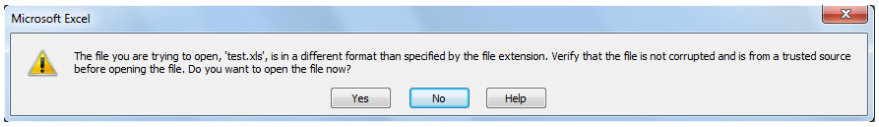
Select a folder where the data will be sent, change the name of the file if desired (a name with the "RP" prefix is entered by default), and press **Save.**

The exportation will not be allowed if an Excel file is opened.



After exportation of the data new parameters of analysis, a new analysis can be performed by choosing different analysis parameter and pressing again the **Analysis** button.

When the exported '.xls' file is opened with Excel 2010 or higher the following message may be shown depending on the version of Excel and the updates installed:



Please ignore this message and click on the 'Yes' button to open the document.

The generated Excel file reports the general information about the session and summarizes the data of each response pattern for further analysis or graphical representation.

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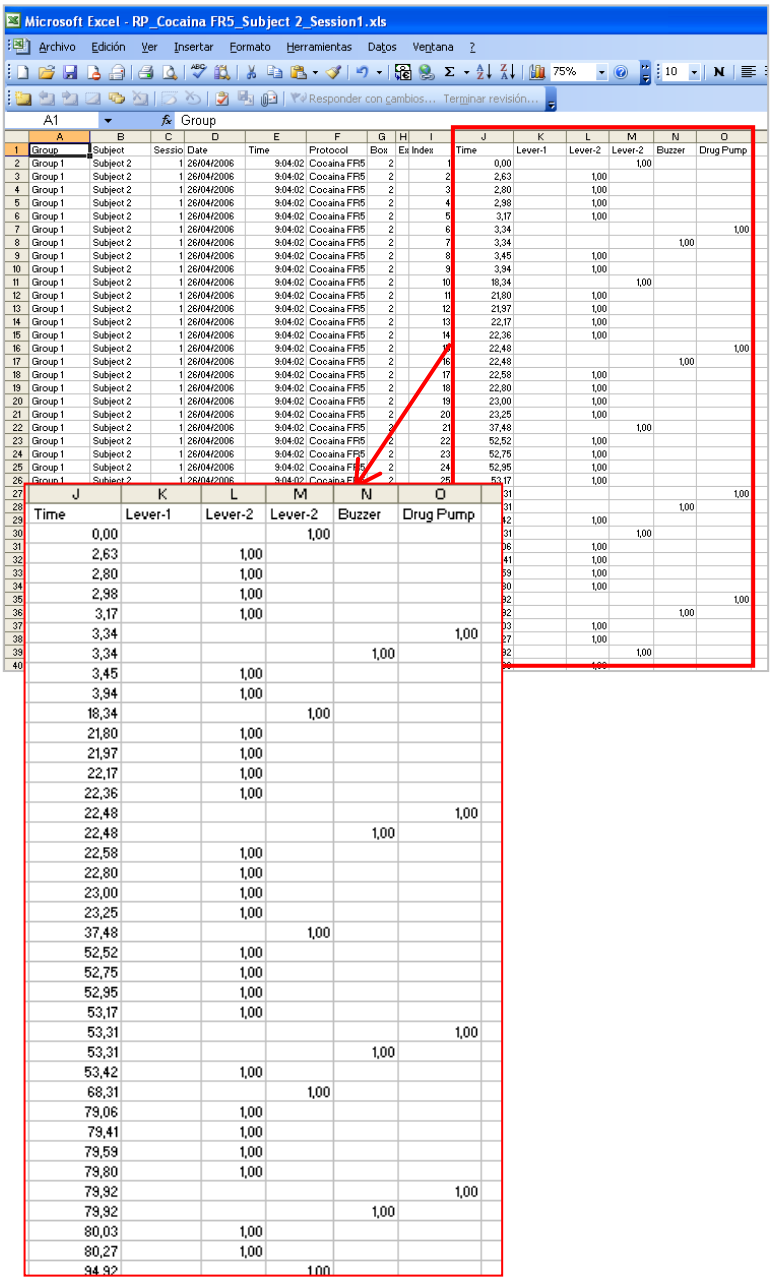

J	K	L	M	N	O
Time	Lever-1	Lever-2	Lever-2	Buzzer	Drug Pump
0,00			1,00		
2,63		1,00			
2,80		1,00			
2,98		1,00			
3,17		1,00			
3,34					1,00
3,34				1,00	
3,45		1,00			
3,94		1,00			
18,34			1,00		
21,80		1,00			
21,97		1,00			
22,17		1,00			
22,36		1,00			
22,48					1,00
22,48				1,00	
22,58		1,00			
22,80		1,00			
23,00		1,00			
23,25		1,00			
37,48			1,00		
52,52		1,00			
52,75		1,00			
52,95		1,00			
53,17		1,00			
53,31					1,00
53,31				1,00	
53,42		1,00			
68,31			1,00		
79,06		1,00			
79,41		1,00			
79,59		1,00			
79,80		1,00			
79,92					1,00
79,92				1,00	
80,03		1,00			
80,27		1,00			
94,92			1,00		

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9.4.2 PACKWIN Freezing Module (CSFR)

9.4.2.1 The Analysis window

The Analysis window of the FREEZING module contains similar options to the ones provided during the data acquisition process:

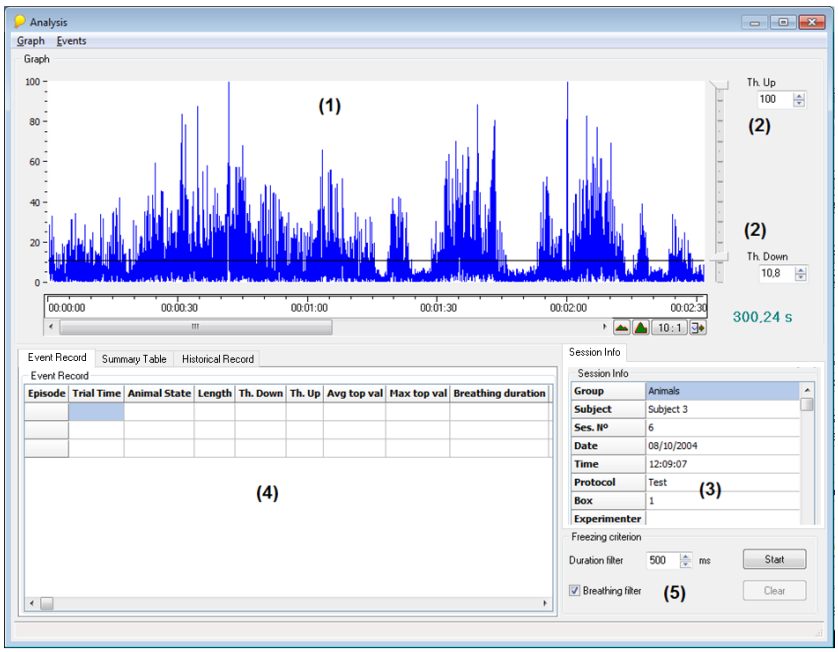
(1) An analogic chart (signal recorded over the time of the experiment).

(2) Two adjustable activity thresholds.

(3) Session details.

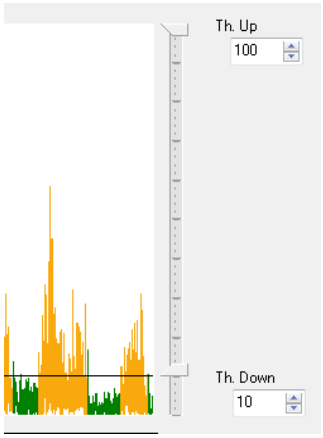
(4) Data tables.

(5) Freezing criteria settings.



9.4.2.2 Set the analysis settings

The Low and high threshold, Freezing criteria and Breathing filter selection can be changed to generate a new set of calculations.



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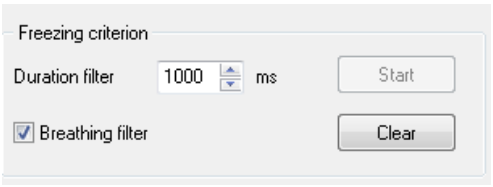
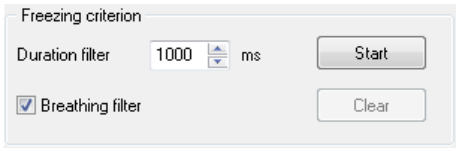


9.4.2.3 Generate the data

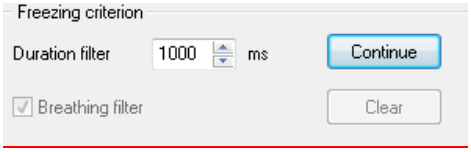
To start re-analysing the signal, press the ***Start*** button.

PACKWIN will recalculate the freezing events according to the new threshold selections and freezing criteria and will display a new analogic signal for data validation.

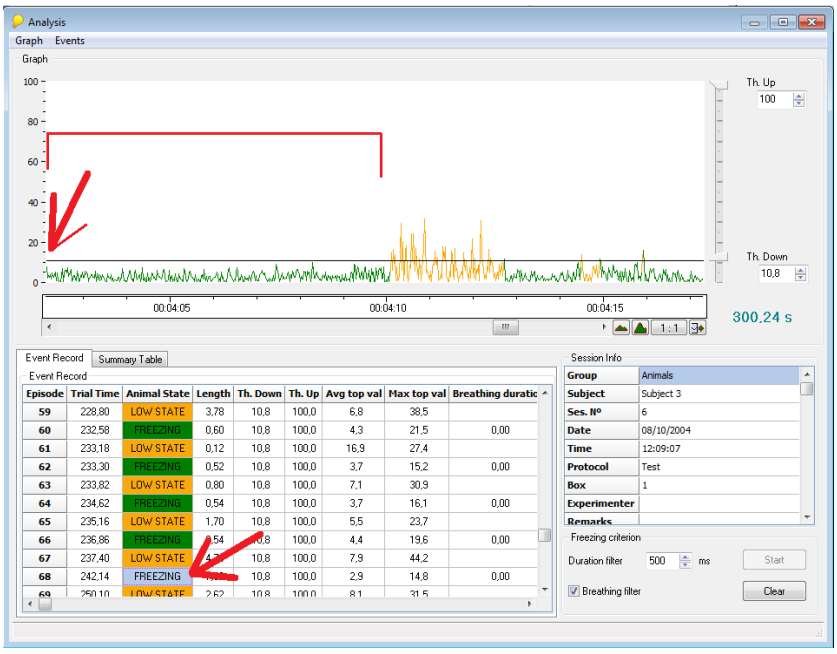


The re-analysis process can be interrupted pressing the ***Pause*** button.

Analysis can be then continued (***Continue***) or re-initialized (***Clear Graph*** and ***Start***).



You may visualize the analogic signal displayed in the raw data table by double-clicking on the corresponding line in the table. The beginning of the events will be displaced to the beginning of the x axis.



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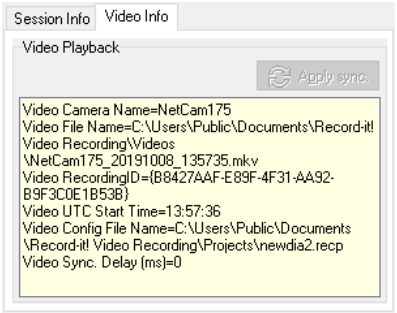
9.4.2.4 Event Record and Summary Table

The Event Record and Summary Tables provided in the main window of the Single Session Analysis are the same ones provided during acquisition. See Chapter 8.3.2.5.4. more detail about the content of these tables for the FREEZING module.

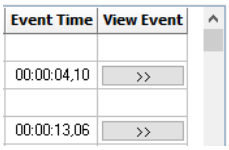
SYNCHRONIZED VIDEO

The Video Recording Info section is shown when the synchronized video recording feature had been used during the data acquisition. A "Video Info" extra tab is added to the "Session Info" section. The "Video Info" will show the details of the synchronized video file recorded during the data acquisition:

- Video Camera Name
- Video File Name
- Video RecordingID
- Video UTC Start Time
- Video Config File Name
- Video Sync. Delay (ms)



This feature adds two new columns to the Event Report:



- **Event Time,** video position in [hh:mm:ss.dd] format.
- **View Event,** seek tool to playback a video-clip starting from the **Event Time** position and during the **Duration** time.

In order to obtain more information about this feature, see chapter o in the Historic Transitions Report for Operant experiments.

9.4.2.5 Historical Record

HISTORICAL RECORD DATA TABLE

The **Historical Record** of the FREEZING module provides a chronological list of all the events (light ON, sound, shock…) occurred during the session. It can be used as a technical report for checking whether the protocol has been executed correctly during the session.

Column title	Description
Index	Consecutive index number.
State	Protocol State when the event occurs.
Time (s)	Time in the experiment (sec).
Description	Description of the event: • Element activation (parameter settings). • State Transition: destination state from source state.
Duration (s)	Duration of the element's status (sec) or the protocol's state (sec).

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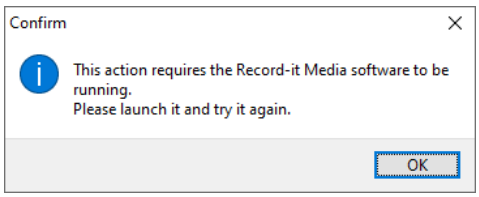
SYNCHRONIZED RECORDED VIDEO

When the session to analyse has a synchronized video file, two more columns are added to the report:

Column title	Description
Event Time	Video Event position (hh:mm:ss.dd).
View Event	Video-Clip player tool.

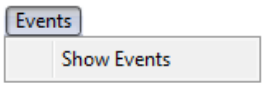
To view recorded video files, RECORD-IT! Media must be running ***BEFORE*** opening the analysis window. The related camera configuration file (*.repc) must also be loaded to allow the Media List to search for the session video file by RecordingID. Otherwise, the full file name and folder will be used (see Record-it! Media user's manual for more information about the Media List and the RecordingID).

PACKWIN will show the following message should the View Event button be selected before RECORD-IT! Media is launched.:



Use the RECORD-IT! Media shortcut to launch the software without having to navigate away from the PACKWIN application.

9.4.2.6 Show Events



The FREEZING module also provides a summary table (Episodes table) containing the number and the duration of the following events occurring for each user-defined interval of time:

- Low threshold events (i.e. episodes of freezing).
- Median threshold events.
- High threshold events.

Select ***Show Events*** in the menu to open the Episodes Table panel.

The **Episodes Table** provides both absolute and accumulated number of episodes/interval time.

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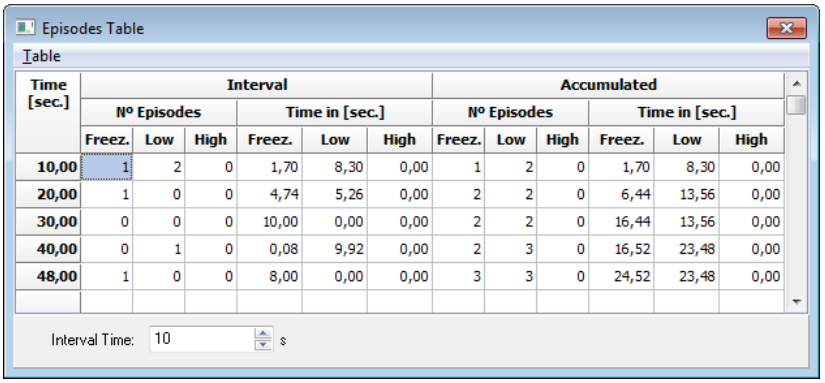
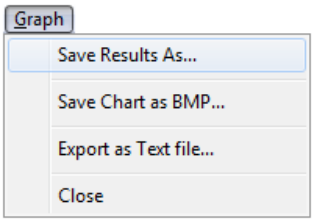


You can specify an interval time before starting the analysis. Just put the desired value into the **Interval time** box. Once chosen, the START button of the main analysis panel has to be pressed for updating the data of the table (if the START button is shown inactivated, press the CLEAR button first).

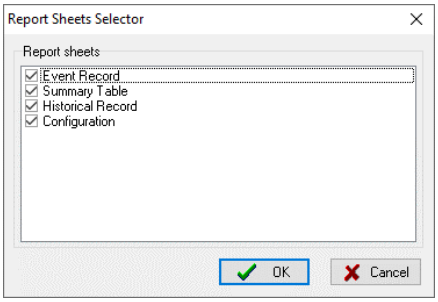


9.4.2.7 Save the Data Tables

Once the analysis process has finished, the raw data obtained from the Event Record, Summary Table and Historical Record can be saved in an Excel file.

Select **Save results As...** from the **Graph** menu.

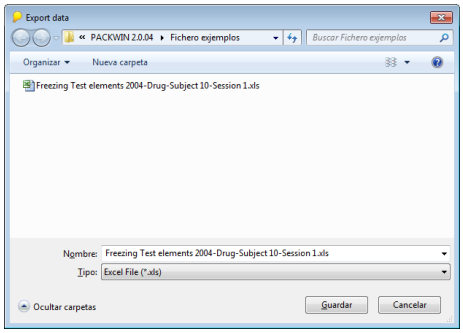
The system will show the **Report Sheets Selector** dialog, where you can select which data tables you want to export.



Select the reports that will be generated and exported and press the **Ok** button.

The system will show the **Export data** dialog, where you can choose the location, the file name, and the type of exportation.

Enter the name of the file, select the format and press the **Save** button.



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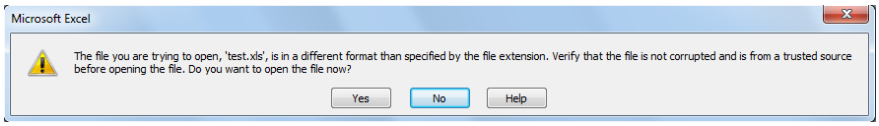
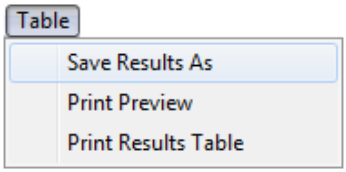
Result tables data are stored in Excel (XLS) format. The different reports will be saved into different sheets on the same workbook.

When the exported '.xls' file is opened with Excel 2010 or higher the following message may be shown depending on the version of Excel and the updates installed:



Please ignore this message and click on the 'Yes' button to open the document.

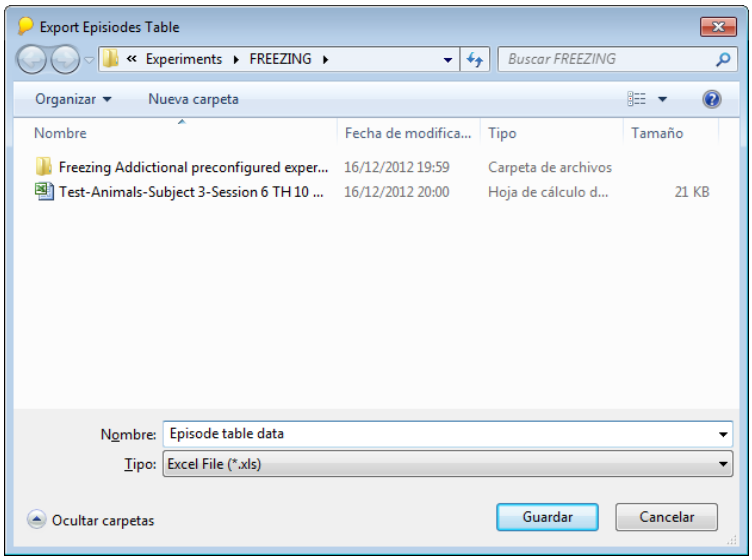
9.4.2.8 Save the Episode table



Select **Save Results As** from the **Table** menu.

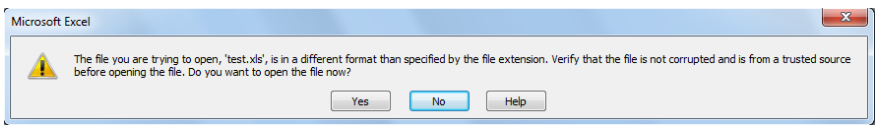
The system will show the **Export data** dialog, where you can choose the location, the file name, and the type of exportation.

Enter the name of the file, select the format and press the **Save** button.



Result tables data are stored in Excel (XLS) format. The different reports will be saved into different sheets on the same workbook.

When the exported '.xls' file is opened with Excel 2010 or higher the following message may be shown depending on the version of Excel and the updates installed:



Please ignore this message and click on the 'Yes' button to open the document.

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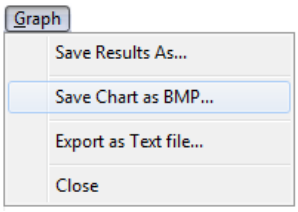
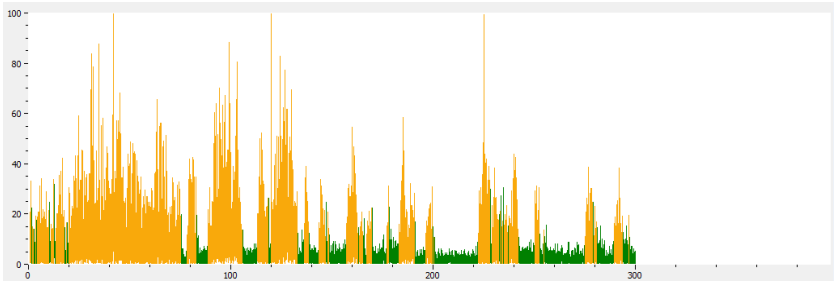
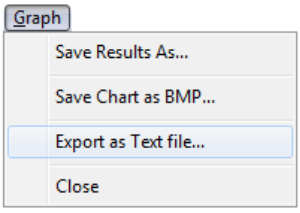


9.4.2.9 Save the graph

For saving the graph as an image, select **Save Chart as BMP...** in the **Graph** menu.

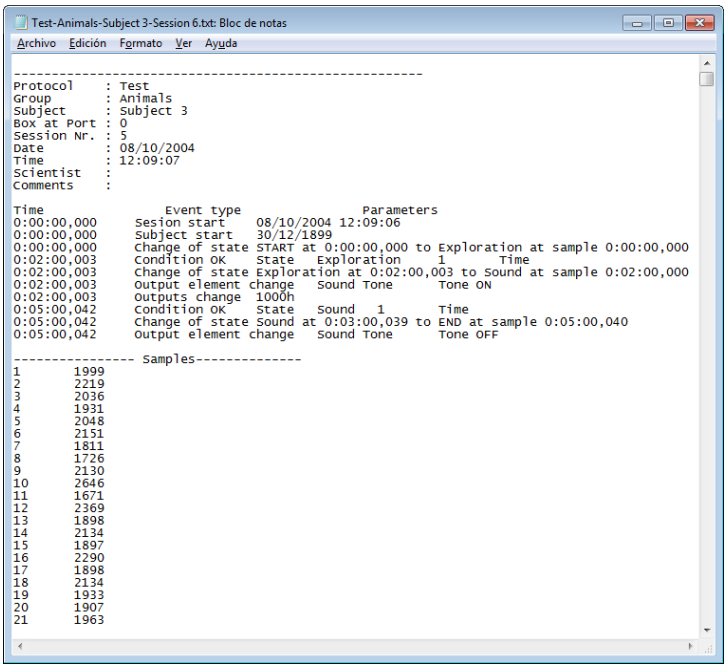




9.4.2.10 Technical data report

The Technical Data report is a text report that shows the internal technical raw data related to the registered session. This report can be asked from our Software Technical Support Department when a support operation is requested by the user.

For generating this report, press the **Export as Text file** option of the **Graph** menu and choose a name and location for the generated file.



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9.4.3 PACKWIN Startle Module (CSST)

9.4.3.1 The Analysis window

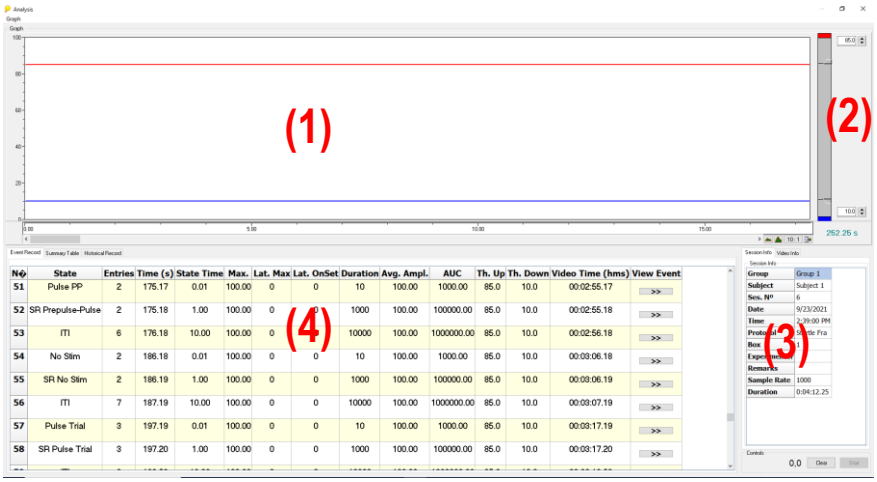
The Analysis window of the STARTLE module contains similar options to the ones provided during the data acquisition process:

(1) An analogic chart (signal = f(time)),

(2) Two adjustable activity thresholds,

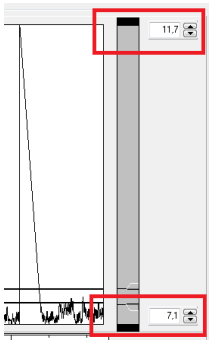
(3) Session details,

(4) Data tables.



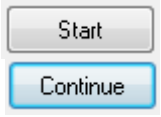
9.4.3.2 Edit the Thresholds

The Low and high threshold can be changed for generating a new set of calculations.



9.4.3.3 Generate the Data

To start re-analysing the signal, press the ***Start*** button.



PACKWIN will recalculate the startle events according to the new threshold selections and display again the analogic signal for data validation.

The re-analysis process can be interrupted pressing the ***Pause*** button.

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Re-analysis can be then continued (***Continue***) or re-initialized (***Clear*** and ***Start***).

To visualize the analogic signal displayed in the raw data table, double-click on the corresponding line in table. The beginning of the events will be displaced to the beginning of the x axis.

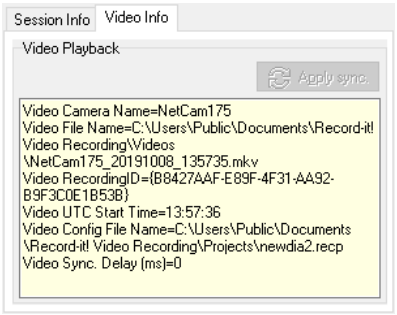
9.4.3.4 Event Record and Summary Table

The Event Record and Summary Tables provided in the main window of the Single Session Analysis are the same that the one provided during acquisition. See Chapter 8.3.2.5.5. for more detail about the content of these tables for the STARTLE module.

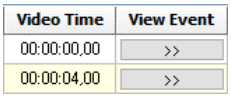
SYNCHRONIZED VIDEO

For data files collected using the synchronized video recording feature, a "Video Info" section is presented in addition to standard "Session Info". Select the "Video Info" tab to view the following details related to the synchronized video recording:

- Video Camera Name
- Video File Name
- Video RecordingID
- Video UTC Start Time
- Video Config File Name
- Video Sync. Delay (ms)



The video recording feature adds two new columns to the Event Report:



- **Event Time,** video position in [hh:mm:ss.dd] format.
- **View Event,** seek tool to playback a video-clip starting from the **Event Time** position and during the **Duration** time. If the **Duration** of the event is 0, playback will not stop until the end of the video is reached.

To obtain more information about this feature, see chapter 0 in the Historic Transitions Report for Operant experiments.

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9.4.3.5 Historical Record

HISTORICAL RECORD DATA TABLE

The **Historical Record** of the STARTLE module provides a chronological list of all the events (light ON, sound, shock…) that have occurred during the session. It can be used as a technical report for checking whether the protocol was executed correctly during the session.

Column title	Description
Index	Consecutive index number.
State	Protocol State when the event occurs.
Time (s)	Time in the experiment (sec).
Description	Description of the event: • Element activation (parameter settings). • State Transition: destination state from source state.
Duration (s)	Duration of the element's status (sec) or the protocol's state (sec).

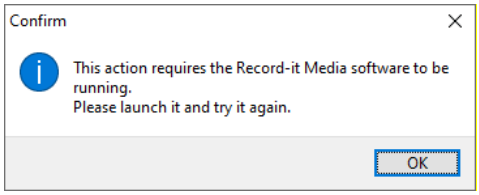
SYNCHRONIZED RECORDED VIDEO

When the analysed session is associated to a synchronized video file, two more columns are added to the report:

Column title	Description
Event Time	Video Event position (hh:mm:ss.dd).
View Event	Video-Clip player tool.

In order to view the recorded video file, the RECORD-IT! Media program must be running ***BEFORE*** opening the analysis window in PACKWIN. Additionally, the related camera configuration file (*.repc) must be loaded to allow the Media List to search for the session video file using the RecordingID. If the configuration file is not selected, the full file name and folder will instead be used (see RECORD-IT! Media user's manual for more information about the Media List and the RecordingID).

If the View Event button is pressed before opening the Record-it! Media software, PACKWIN will show this message:



Use the RECORD-IT! Media shortcut in order to run RECORD-IT! Media without having to navigate away from the PACKWIN application.

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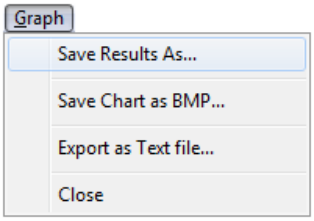
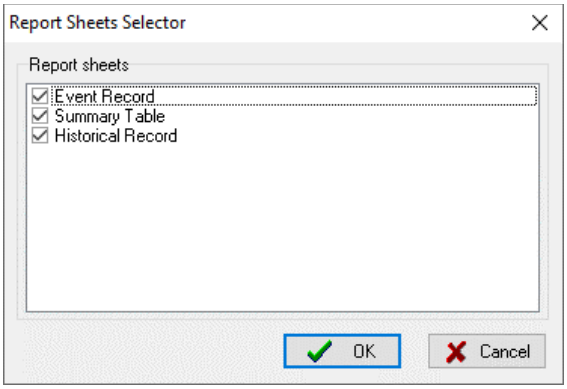


9.4.3.6 Save the Data Tables

Once the analysis process has finished, the raw data obtained from the Event Record, Summary Table, and Historical Record can be saved in an Excel file.

Select **Save Results As...** from the **Graph** menu.

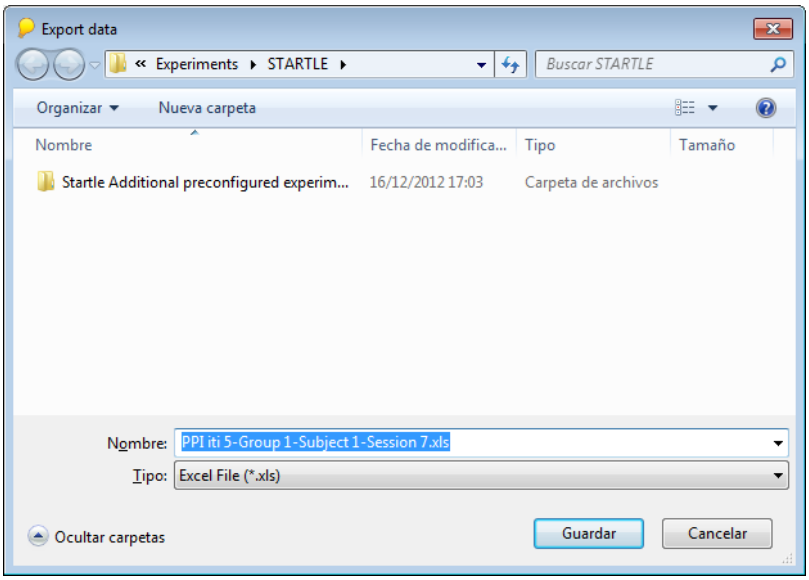
The system will show the **Report Sheets Selector** dialog, where you can select which data tables you want to export.



Select the reports that will be generated and exported and press the **Ok** button.

The system will show the **Export data** dialog, where you can choose the location, the file name, and the type of exportation.

Enter the name of the file, select the format and press the **Save** button.



Result tables data are stored in Excel (XLS) format. The different reports will be saved into different sheets on the same workbook.

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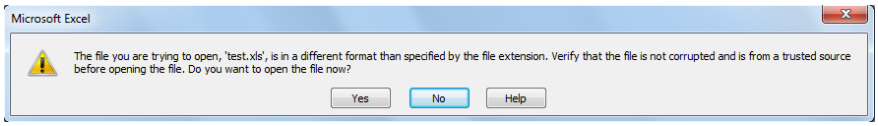
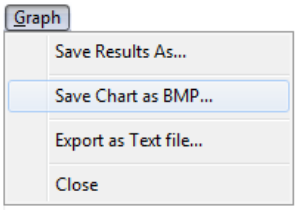
When the exported '.xls' file is opened with Excel 2010 or higher the following message may be shown depending on the version of Excel and the updates installed:

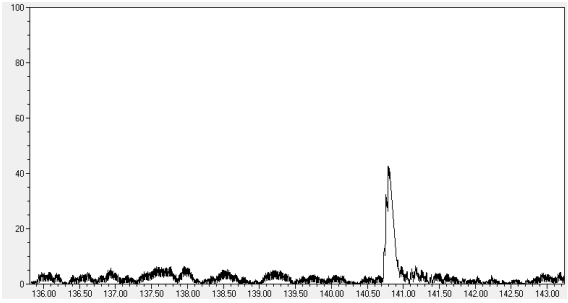


Please ignore this message and click on the 'Yes' button to open the document.

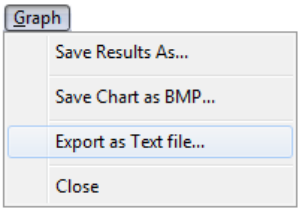
9.4.3.7 Save the Graph



To save the graph as an image, select **Save Chart as BMP...** in the **Graph** menu.

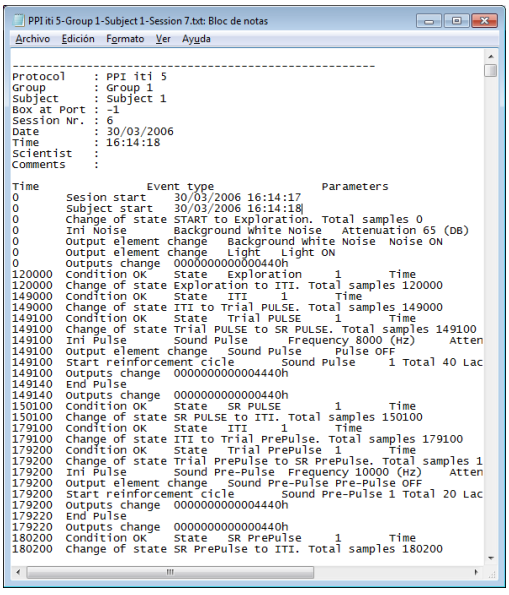


9.4.3.8 Technical Data Report



The Technical Data report is a text report that shows the internal technical raw data related to the registered sessions. This report can be asked from our Software Technical Support Department when a support operation is requested by the user.

For generating this report, press the **Export as Text file** option of the **Graph** menu and choose a name and location for the generated file.



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9.5 Generate a Multi-session analysis report (batch analysis)

The PACKWIN batch analysis tool allows the generation of data reports simultaneously for a batch of sessions selected by the user in the session table.

Batch analysis is arranged in the following steps:

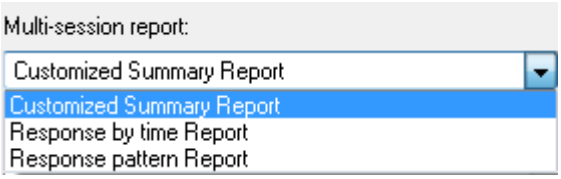
- Choosing a Report.
- Selecting and configuring the data to be included in the reports.
- Data Previewing.
- Data exportation to Excel and/or to an image file.

These steps are specific to the modules licensed and to the activated experiment modules (CS, HO, VT, CSFR, CSST).

9.5.1 PACKWIN Operant Module (CS)

9.5.1.1 Chose a multi-session report

Choose a report from the available list of reports in the Multi-session report section.



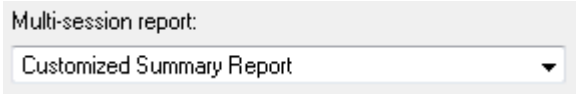
PACKWIN provides 3 customized reports:

- the Customized Summary Report
- the Response by time Report
- the Response pattern Report

9.5.1.2 Customized Summary Report

The **Customized Summary Report** provides basic data related to the animal response, reinforcement given, and a great number of other operant behaviour related calculations. The report is completely configurable: the user can select and configure the calculations that will be reported.

Choose the Customized Summary Report option in the **Multi-session** report section.



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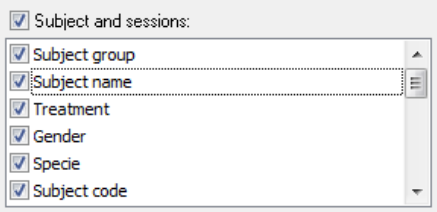
DATA SELECTION

Select the data and calculation that will be shown in the reports.

Select Subject and sessions data

- Select the **Subject and Session** information to be included in the report.



- To select an item, check the associated box ☑.
- To unselect an item, uncheck the associated box ☐.
- To select or unselect all the items, check or uncheck the **Subject and sessions** title box.

The available options are:

Subject group	**Name of the group associated to the subject (user-defined)**
Subject name	Name of the subject (user-defined)
Treatment	Treatment (user-defined)
Gender	Animal Gender (user-defined)
Species	Animal species (user-defined)
Subject code	Subject code (internal number)
Group code	Group code (internal number)
Date	Date of session registering
Clock time	Time of session registering
Experiment file	Name of the experiment file containing the session
Remarks	Remarks (user-defined)
Box type	Box type associated to the protocol used in the registered session
Box number	Number of the box in which the session has be registered
Yoked procedure	Yoked status of the box (Independent, Master or Slave)
Protocol	Protocol used in the registered session
Session number	Number of session for the animal used in the session
Session duration	Full duration of the registered session
Start interval	Start time set for analysis
End interval	End time set for analysis
Split time	Split time set for analysis

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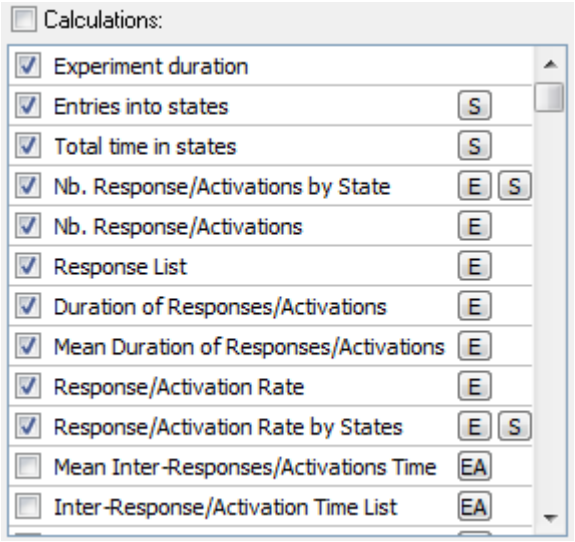
Select the calculations to be reported in the report



- To select a calculation, check the associated box ☑.
- To unselect a calculation, uncheck the associated box ☐.
- To select or unselect all the calculations, check or uncheck the **Calculations** title box.
- Some Calculation can be filtered by States S or by the Elements E of the box.
- Some Calculations need Elements EA or States SA associations to be configured.

The available calculations are:

Calculation	**Meaning**
Experiment duration	Duration of experiment, taking into account the **Time selection** settings (Start time, End time).
Entries into states	Total number of entries into each state. States can be filtered using the S filter.
Total time in states	Accumulated time elapsed into each state. The States can be filtered using the S filter.
Nb. Response/Activations by State	Number of responses or element activation registered in each state. The Responses and States can be filtered using the E and the S filters.
Nb. Response/Activations	Total number of response or element activation registered during the session. The Elements can be filtered using the E filter.

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Calculation	Meaning
Response List	Table listing the registered responses with respective starting time and duration. The Responses can be filtered using the E filter.
Duration of Response/Activations	Accumulated duration of response or element activation registered during the session. The Elements can be filtered using the E filter.
Mean Duration of Responses/Activations	Mean duration of response or element activation registered during the session. The Elements can be filtered using the E filter.
Responses/Activations Rate	Response or element activation rate registered during the session. The Elements can be filtered using the E filter.
Responses/Activations Rate by States	Response or element activation rate registered for each state during the session. The Elements and States can be filtered using the E and the S filters.
Mean Inter-Responses/Activation Time	Mean of all the intervals of time between consecutives responses selected by the user in the Elements Association EA panel. This option is unchecked by default because it requires the user defining Elements Associations.
Inter-Response/Activations Time	Table listing the detail of the times between responses used for calculation the Mean Inter-responses/activation time. This option is unchecked by default; it requires the user defining Elements Association EA panel.
Mean Inter-States Time	Mean of the times calculated between the states selected by the user. This option is unchecked by default; it requires the user defining States Association SA panel.
Inter-State Time List	Table listing the detail of the times between states used for calculation the Mean Inter-state time. This option is unchecked by default; it requires the user defining States Association SA panel.
Breaking point/Last ration	Number of time the subject has to perform the selected response in the current state for inducing a change to the next state. It represents an index of animal motivation in progressive ratio experiments

The elements of the cage and the state of the protocols to which the calculation will be applied can be filtered using the following filters:

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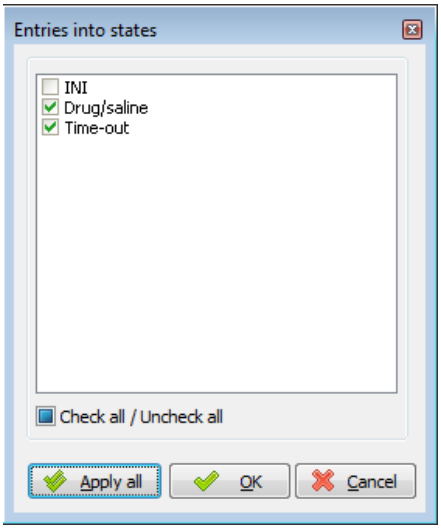
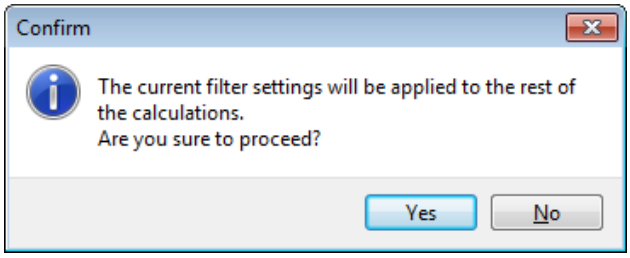
Apply a state filter

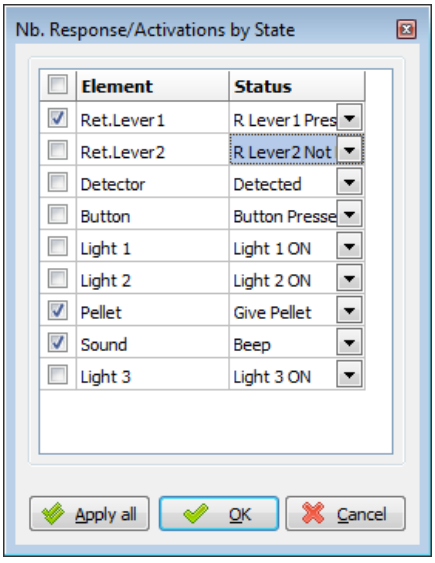


- To select a state, check the associated box ☑.
- To unselect a state, uncheck the associated box ☐.
- Several states can be chosen.

Use the **Apply all** button to apply the configuration to all the calculations using the State filter.



Apply an element filter



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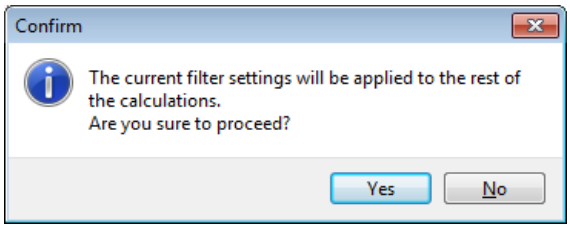
- To select an element, check the associated box ☑.
- To unselect an element, uncheck the associated box ☐.
- For each element, the status of the elements to take into account can be chosen by using the available list provided in the **Status** column.
- To select or unselect all the elements, check or uncheck the title box.

Use the **Apply all** button to apply the configuration to all the calculations using the Element filter.

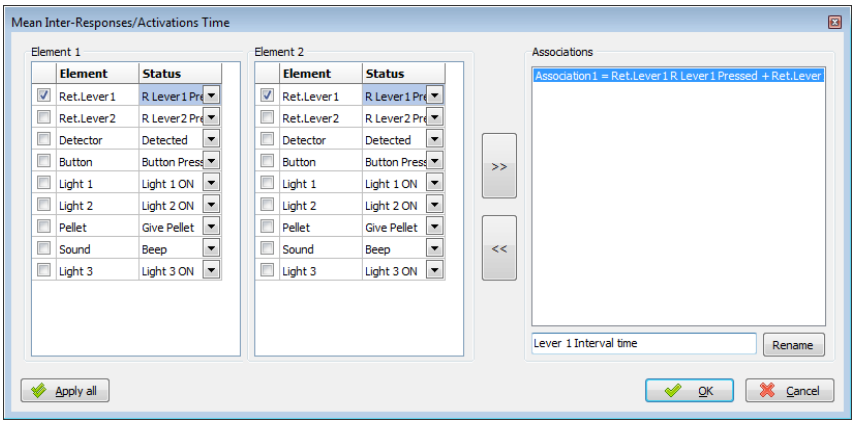


Set an element Association filter

The Element Association filter is used to define the association of two elements that will be used in the calculation of the mean inter-response time and for generating the inter-response list.

In these calculations, the report will calculate the time between the consecutive occurrences of the two elements defined in the Element 1 and Element 2 tables.

As an example, an association can be configured to calculate the interval time between two consecutives responses on the active lever (here, Lever 1/Pressed status).



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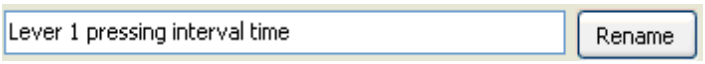
To define an Element Association:

- Select an element and its status in the Element 1 table. Only one element can be chosen.
- Select an element and its status in the Element 2 table. Only one element can be chosen.
- Press the Right-directed double arrows button for associating the two selected elements.
- Press the Left-directed double arrows button for disassociating created elements associations.
- Enter a name for defining the association and press the **Rename** button for applying the configuration.



Several associations can be created in the Element Association panel; the calculations will be made for each created association.

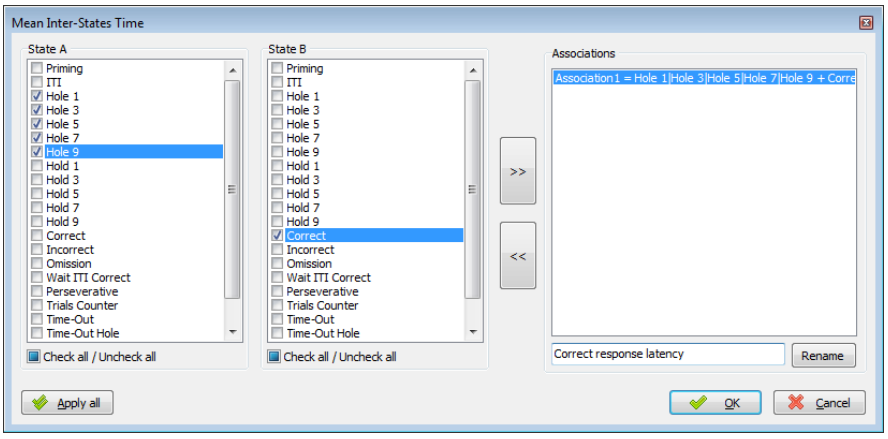
Use the **Apply all** button for applying the configuration to all the calculations using associations of elements.

Set a state Association filter

The State Association filter is used to define the association of two states that will be used in the calculation of the mean inter-state time and for generating the inter-state list.

In these calculations, the report will calculate the time between the consecutive initiation of the two states defined in the **State A** and **State B** tables.

As an example, in a Nine-hole 5CSRT task, the user can configure an association to calculate the Correct response latency by calculating the interval time between initiation of the States of the protocol in which the LED are switched ON and the entry into the Correct state (see next table)



To define a State Association:

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- Select one of several states in the States 1 table. Several States can be chosen.
- Select a State in the State 2 table. Only one state can be chosen.
- Press the Right-directed double arrows button for associating the two selected states.
- Press the Left-directed double arrows button for disassociating created states associations.
- Enter a name for defining the association and press the **Rename** button for applying the configuration.

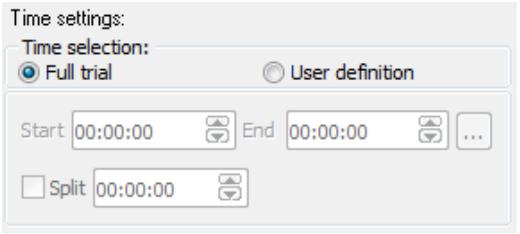
Several associations can be created in the State Association panel; the calculations will be made for each created association.

Use the **Apply all** button for applying the configuration to all the calculations using associations of states.

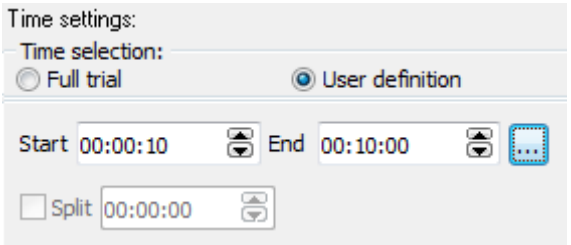
TIME SELECTION

In the Customized Summary report, the interval time of analysis can be defined:

- Full session: the calculations will be applied to the entire registered session.



- User-defined: the user can define a new interval of analysis by setting a new **Start** and **End** time both in hh:mm:ss unit.



By pressing the button, the duration of the longer session will be displayed as the **End** time value.

The value of the **End** time has to be strictly higher than the value of the **Start** time.

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In the Customized Summary report the **Split** option is not available.

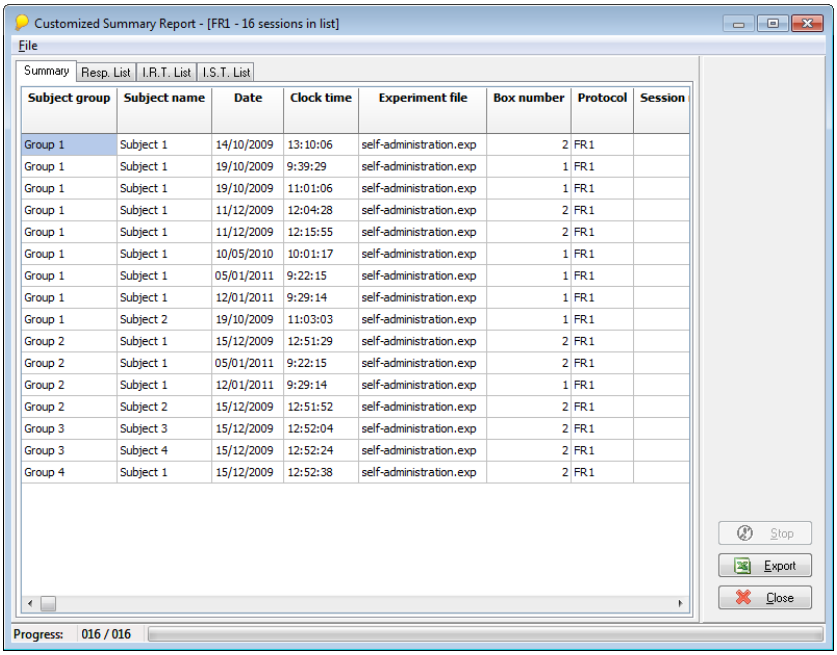
The time selection will only apply to the selected sessions highlighted in blue in the session table.

DATA REPORT PREVIEW

Press the **Analyse…** button to preview the data reports.

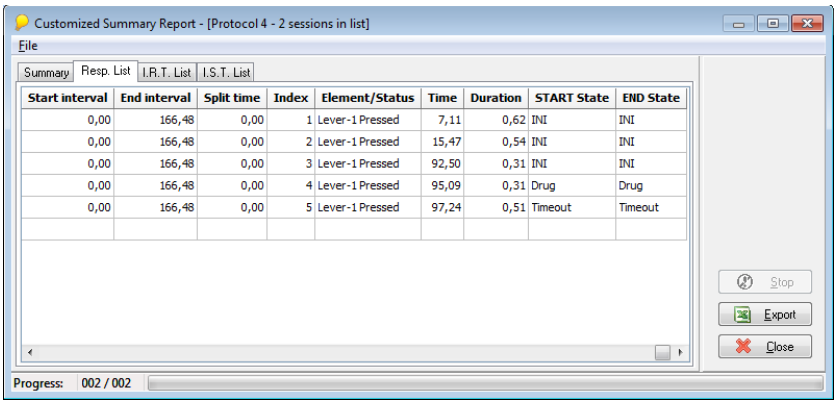
The data are presented into 4 different tabs:

Summary table (1 row by session) containing all the Subject & Session items and calculations previously selected.



Subject group	Subject name	Date	Clock time	Experiment file	Box number	Protocol	Session
Group 1	Subject 1	14/10/2009	13:10:06	self-administration.exp	2	FR1	
Group 1	Subject 1	19/10/2009	9:39:29	self-administration.exp	1	FR1	
Group 1	Subject 1	19/10/2009	11:01:06	self-administration.exp	1	FR1	
Group 1	Subject 1	11/12/2009	12:04:28	self-administration.exp	2	FR1	
Group 1	Subject 1	11/12/2009	12:15:55	self-administration.exp	2	FR1	
Group 1	Subject 1	10/05/2010	10:01:17	self-administration.exp	1	FR1	
Group 1	Subject 1	05/01/2011	9:22:15	self-administration.exp	1	FR1	
Group 1	Subject 1	12/01/2011	9:29:14	self-administration.exp	1	FR1	
Group 1	Subject 2	19/10/2009	11:03:03	self-administration.exp	1	FR1	
Group 2	Subject 1	15/12/2009	12:51:29	self-administration.exp	2	FR1	
Group 2	Subject 1	05/01/2011	9:22:15	self-administration.exp	2	FR1	
Group 2	Subject 1	12/01/2011	9:29:14	self-administration.exp	1	FR1	
Group 2	Subject 2	15/12/2009	12:51:52	self-administration.exp	2	FR1	
Group 3	Subject 3	15/12/2009	12:52:04	self-administration.exp	2	FR1	
Group 3	Subject 4	15/12/2009	12:52:24	self-administration.exp	2	FR1	
Group 4	Subject 1	15/12/2009	12:52:38	self-administration.exp	2	FR1	

Resp. List table generated from the **Response List** calculation and containing the list of activation or deactivation of the requested elements (1 row for each element/status initialization detection) with the number of event (**Index**), its time of initiation (**Time**), its duration (**Duration**), the state of the protocol in which the selected element status has been initiated (**START State**), and the state of the protocol in which the selected element status has been finalized (**END State**).



Start interval	End interval	Split time	Index	Element/Status	Time	Duration	START State	END State
0,00	166,48	0,00	1	Lever-1 Pressed	7,11	0,62	INI	INI
0,00	166,48	0,00	2	Lever-1 Pressed	15,47	0,54	INI	INI
0,00	166,48	0,00	3	Lever-1 Pressed	92,50	0,31	INI	INI
0,00	166,48	0,00	4	Lever-1 Pressed	95,09	0,31	Drug	Drug
0,00	166,48	0,00	5	Lever-1 Pressed	97,24	0,51	Timeout	Timeout

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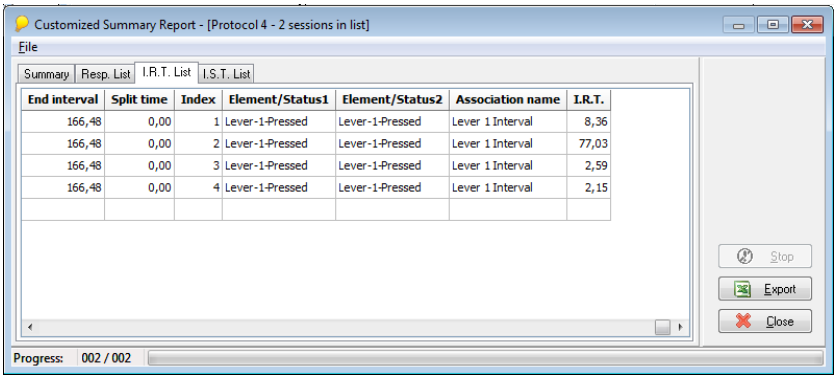
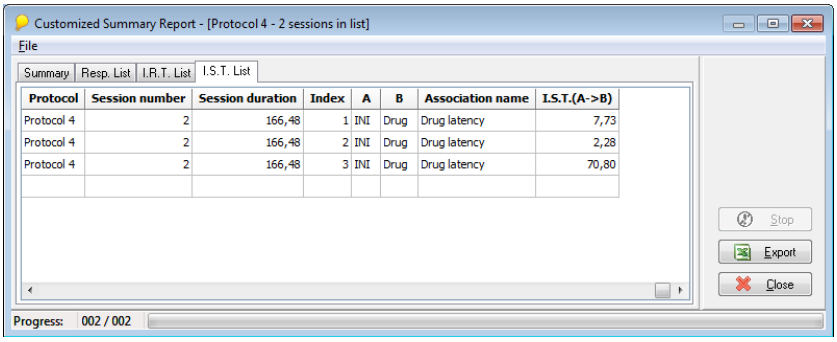
I.R.T. List: table generated from the **Inter-Response/Activation Time List** calculation. Contains the list of the interval of time between the selected Elements/Status in each Element association (1 row for each element/status association detection) with the number of the association occurrences (**Index**), the name and status of the Element 1 of the association (**Element/Status1**), the name and the status of the Element 2 of the association (**Element/Status2**), the name of the element association (**Association name),** and the Inter-response Time value for each association detected (**I.R.T.**).



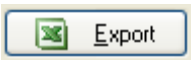
End interval	Split time	Index	Element/Status1	Element/Status2	Association name	I.R.T.
166,48	0,00	1	Lever-1-Pressed	Lever-1-Pressed	Lever 1 Interval	8,36
166,48	0,00	2	Lever-1-Pressed	Lever-1-Pressed	Lever 1 Interval	77,03
166,48	0,00	3	Lever-1-Pressed	Lever-1-Pressed	Lever 1 Interval	2,59
166,48	0,00	4	Lever-1-Pressed	Lever-1-Pressed	Lever 1 Interval	2,15

I.S.T. List: table generated from the **Inter-State Time List** calculation. Contains the list of the interval of time between the selected States in each State association (1 row for each state association detection) with the number of the association occurrences (**Index**), the name of the State A of the association (**A**), the name of the State B of the association (**B**), the name of the state association (**Association name),** and the Inter-state Time value for each association detected (**I.S.T.**).



Protocol	Session number	Session duration	Index	A	B	Association name	I.S.T.(A->B)
Protocol 4	2	166,48	1	INI	Drug	Drug latency	7,73
Protocol 4	2	166,48	2	INI	Drug	Drug latency	2,28
Protocol 4	2	166,48	3	INI	Drug	Drug latency	70,80

DATA EXPORTATION



Press the **Export** button to export the tables directly in Excel format.

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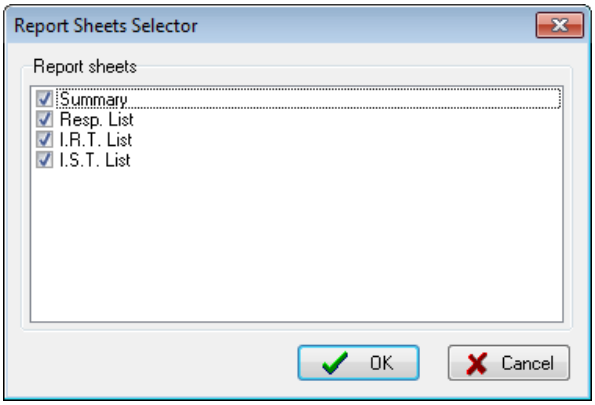
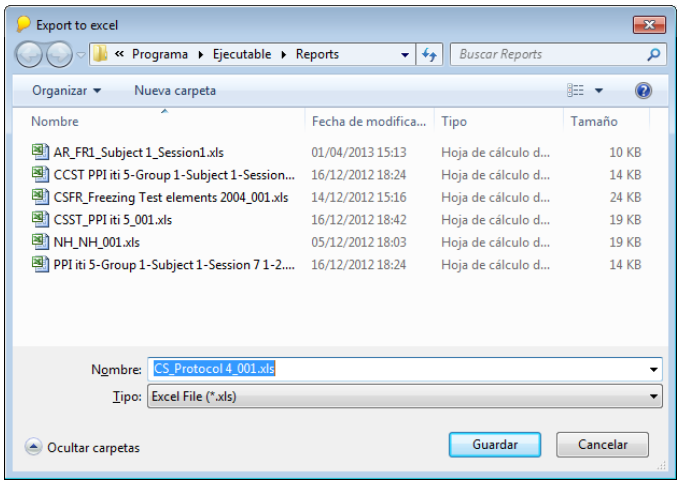


The user can select the data tables to export between the 4 available tables by checking the corresponding check box and pressing the **OK** button.



The Excel file name and location can also be defined by the user in the **Export to excel** saving panel.

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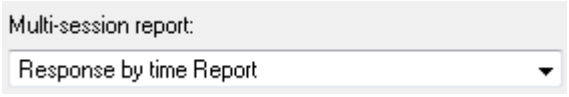
9.5.1.3 Response by Time report

The **Response by Time** provides an analysis of the response registered during the session split by intervals of time.

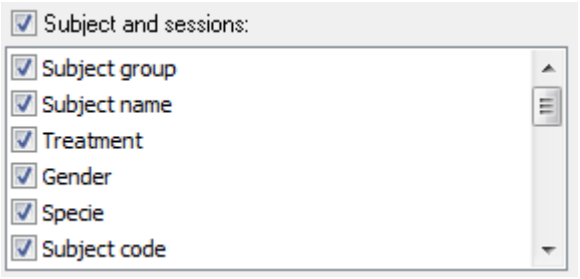
Choose the **Response by Time** Report option in the **Multi-session** report section.



DATA SELECTION

Select Subject and session data

Select the **Subject and Session** information to be reported in the report.



The options available are the same than in the Customized Summary Report (see chapter 9.5.1.2.1.).

Select the Calculations to be reported in the report

In the Response by Time report, the only calculation available is the Nb. Response/Activation which will provide the total number of responses registered for each defined interval of time. The Elements can be filtered using the same E filter that was used in the Customized Summary report (see chapter 9.5.1.2.1.).

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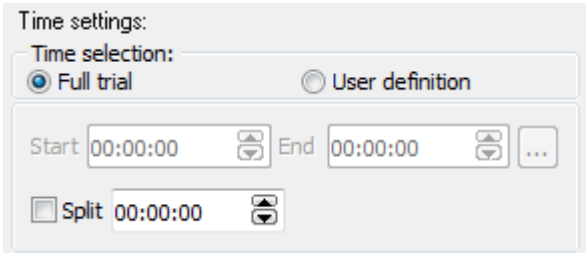
TIME SELECTION

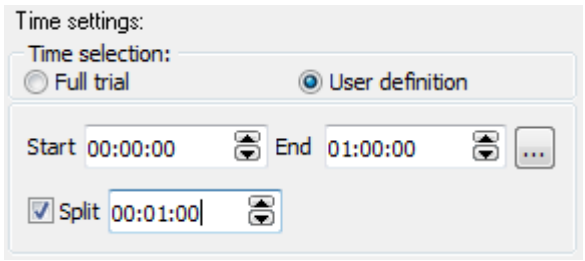
In the **Response by Time** report, all the options of the Time selection section are available

Select the interval time of analysis:

- Full session: the calculations will be applied to the entire registered session.



- User-defined: the user can define a new interval of analysis by setting a new **Start** and **End** time both in hh:mm:ss unit.



By pressing the button, the duration of the longer session will be displayed as the **End** time value.

The value of the **End** time has to be higher than the value of the **Start** time.

A splitting time (**Split,** hh:mm:ss) can also be defined for calculating the number of responses for each fixed subinterval of time.

The time selection will only apply to the selected sessions highlighted in blue in the session table.

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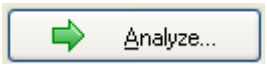
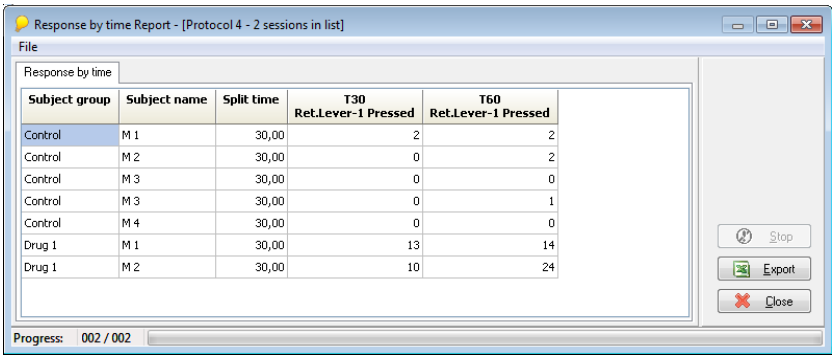
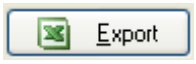


DATA REPORT PREVIEW

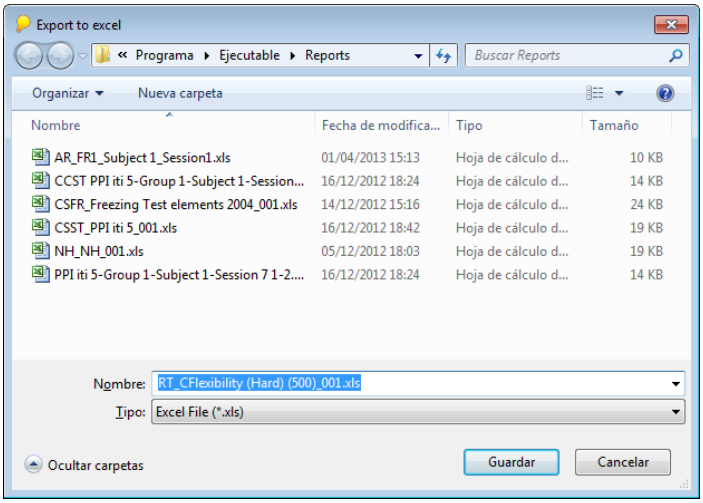
Press the **Analyse...** button to preview the data reports.



DATA EXPORTATION



Press the **Export** button to export the tables directly in Excel format.

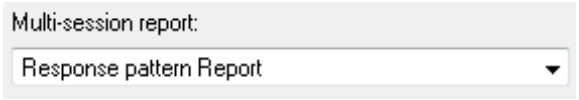


The Excel file name and location can also be defined by the user in the **Export to excel** saving panel.

9.5.1.4 Response Pattern report

The **Response pattern report** provides a graphical representation of the temporal distribution of the user-selected responses.

Choose the **Response Pattern Report** option in the **Multi-session** report section.



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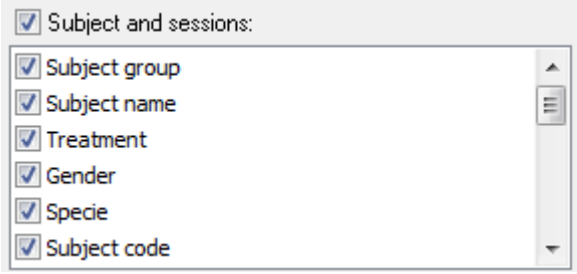
DATA SELECTION

Select Subject and session data

Select the **Subject and Session** information to be reported in the report.



The options available are the same than in the Customized Summary Report (see chapter 9.5.1.2.1.).

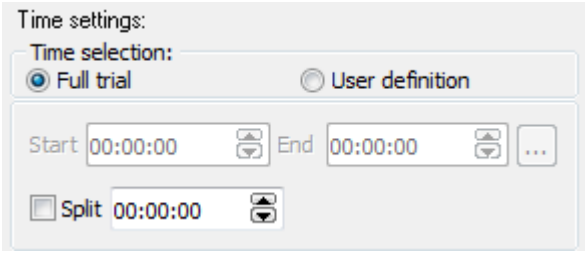
Select the Calculations to be reported in the report

In the Response Pattern report, the only calculation available is the Nb. Response/Activation. The Elements can be filtered using the same E filter that the one used in the Customized Summary report (see Customized Summary chapter). Only one response can be chosen and will be represented in one diagram for each experimental group.

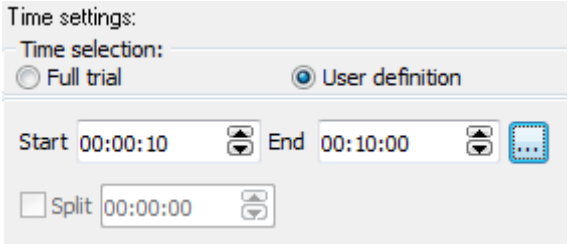
TIME SELECTION

In the Response Pattern report, the interval time of analysis can be defined:

- Full session: the calculations will be applied to the entire registered session.



- User-defined: the user can define a new interval of analysis by setting a new **Start** and **End** time both in hh:mm:ss unit.



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By pressing the … button, the duration of the longer session will be displayed as the **End** time value.

The value of the **End** time has to be strictly higher than the value of the **Start** time.

In the Response Pattern report the **Split** option is not available.

The time selection will only apply to the selected sessions highlighted in blue in the session table.

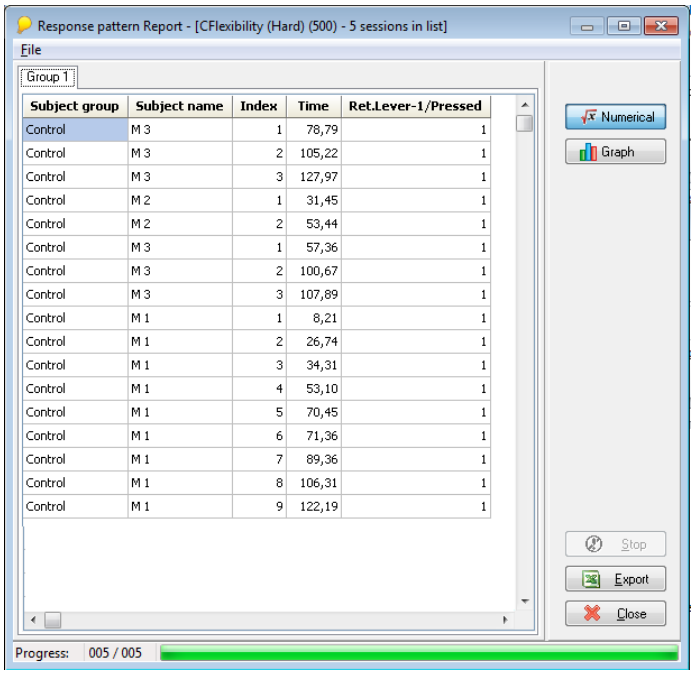
DATA REPORT PREVIEW

Press the **Analyse…** button to preview the reports.

The data preview panel provides two kinds of reports: Numerical and Graph reports.

Numerical report

The numerical report provides one table of data for each experimental group used in the selected sessions. Each table is presented in a different tab (Here, Control and Drug 1 tabs)



Subject group	Subject name	Index	Time	Ret.Lever-1/Pressed
Control	M 3	1	78,79	1
Control	M 3	2	105,22	1
Control	M 3	3	127,97	1
Control	M 2	1	31,45	1
Control	M 2	2	53,44	1
Control	M 3	1	57,36	1
Control	M 3	2	100,67	1
Control	M 3	3	107,89	1
Control	M 1	1	8,21	1
Control	M 1	2	26,74	1
Control	M 1	3	34,31	1
Control	M 1	4	53,10	1
Control	M 1	5	70,45	1
Control	M 1	6	71,36	1
Control	M 1	7	89,36	1
Control	M 1	8	106,31	1
Control	M 1	9	122,19	1

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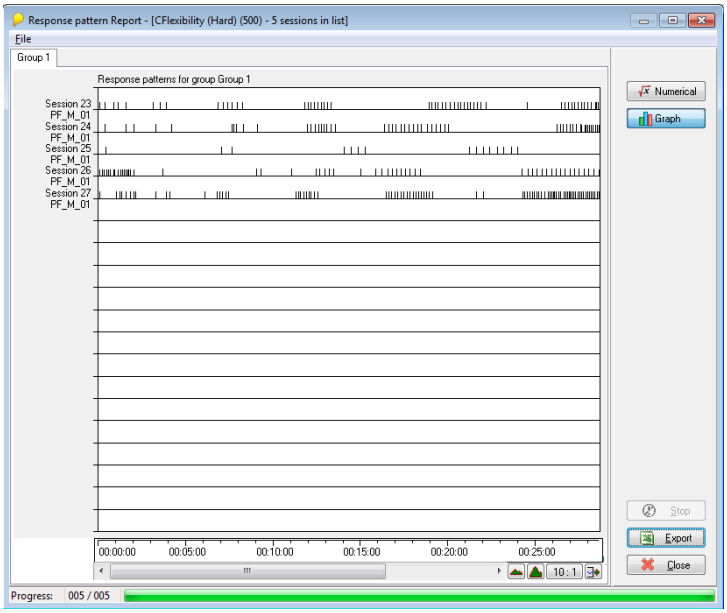
Graph report

The Graph report provides a diagram for each experimental group used in the selected sessions. Each diagram is presented in a different tab (Here, Control and Drug 1 tabs)

A unique X axis shows the session acquisition time (hh:mm:ss). Y axis represents the sessions related to each experimental group.

The occurrence of the responses is represented by a black vertical line.



A time horizontal scroll is available by displacing the horizontal scroll bar located just below X-time axis.

The X-time axis is provided of a zoom-in and zoom-out options, using respectively the and buttons located at the bottom right side of the signal. The zoom list can be also used.

Zoom is only applied in the time scale (X axis).

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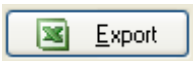
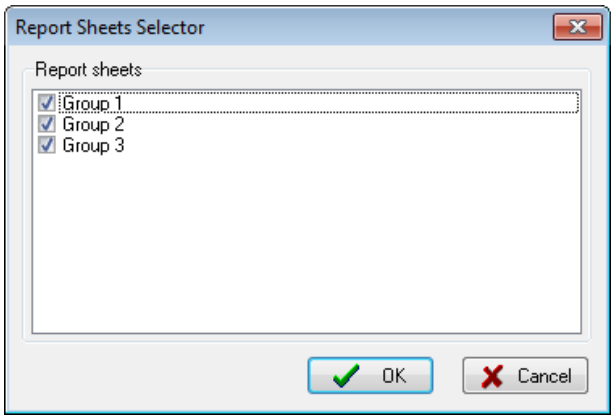
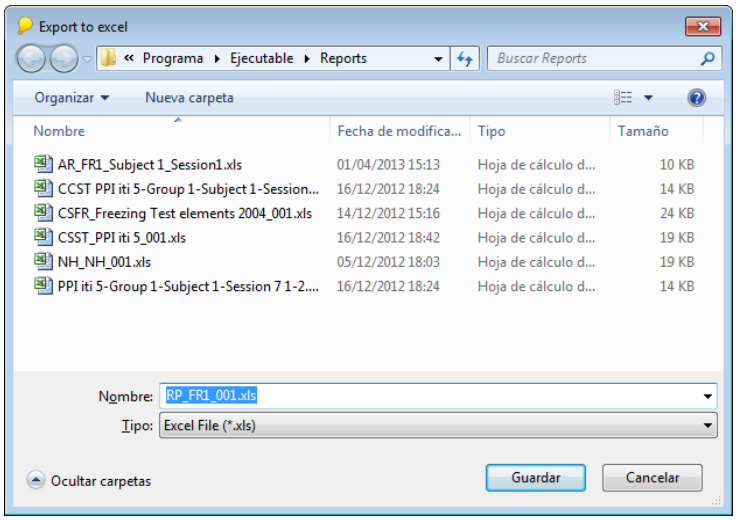
DATA EXPORTATION

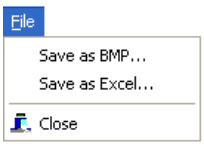


Press the **Export** button for exporting the Numerical tables directly in Excel format. The exported tables can be filtered using the Report Sheets Selector panel.



The Excel file name and location can also be defined by the user in the **Export to excel** saving panel.





Use the **Save as BMP...** option of the **File** menu for saving the Graph as an image file. Only the image of the selected tag will be exported.

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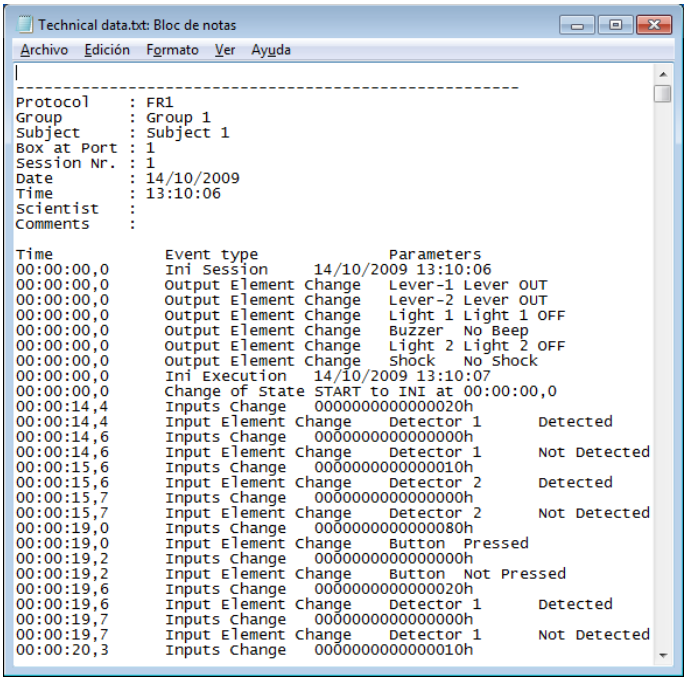
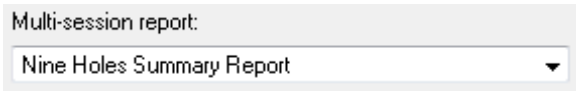
9.5.1.5 Technical data report

The Technical Data report is a text report that shows the internal technical raw data related to the registered sessions. This report can be asked from our Software Technical Support Department when a support operation is requested by the user.

To generate this report, press the **Export to Txt** button and choose a name and location for the generated file.

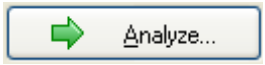


9.5.2 PACKWIN Operant Nine Hole Module (HO)



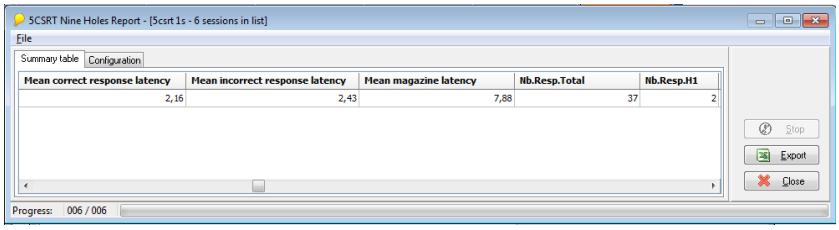
PACKWIN provides a specific **Nine Holes Summary Report** whose contents depends on the associated Assistant tools (Magazine & Hole Training or 5-CSRT task procedures).

The reports are predefined, so no Subject & Session, Calculation, or Time selection configuration is available.



Press the **Analyse** button to preview the data tables before their exportation to Excel.

Data is presented into 2 different tabs: Summary table and Configuration (see tables bellow for the detail of the content of each report).



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9.5.2.1 Magazine & Training Report

Column	Nine Hole Box type	Nine Hole Nine Pellets Box type
Subject group	Name of the group associated to the subject (user-defined)	
Subject name	Name of the subject (user-defined)	
Treatment	Treatment (user-defined)	
Gender	Animal Gender (user-defined)	
Species	Animal species (user-defined)	
Subject code	Subject code (internal number)	
Group code	Group code (internal number)	
Protocol	Protocol name (user-defined)	
Experiment duration	Time spent between the START and END state	
Nb. Reinforcements	Number of entries into the Reinforcement state	
Nb. Detections	Total number of animal detections in the food holder	Not Available
Mean magazine latency	Mean duration of time spent between the initiation of the "reinforcement" state and the "ITI" when the "Wait animal detection in food holder to allow next reinforcement" is checked. If the option is not checked, the case is not filled.	Not Available
Nb. Resp. Total	Total number of nose pole into the Holes	
Nb. Resp. H1	Total number of nose poke detected in the Hole 1	
Nb. Resp. H2	Total number of nose poke detected in the Hole 2	
Nb. Resp. H3	Total number of nose poke detected in the Hole 3	
Nb. Resp. H4	Total number of nose poke detected in the Hole 4	
Nb. Resp. H5	Total number of nose poke detected in the Hole 5	
Nb. Resp. H6	Total number of nose poke detected in the Hole 6	
Nb. Resp. H7	Total number of nose poke detected in the Hole 7	
Nb. Resp. H8	Total number of nose poke detected in the Hole 8	
Nb. Resp. H9	Total number of nose poke detected in the Hole 9	

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9.5.2.2 5-CSRT task Report

Column	Nine Hole Box type	Nine Hole Nine Pellets Box type
Subject group	Name of the group associated to the subject (user-defined)	
Subject name	Name of the subject (user-defined)	
Treatment	Treatment (user-defined)	
Gender	Animal Gender (user-defined)	
Species	Animal species (user-defined)	
Subject code	Subject code (internal number)	
Group code	Group code (internal number)	
Protocol	Protocol name (user-defined)	
Nb. Trials	Total number of trials	
Experiment duration	Time spent between the START and END state	
Nb. Reinforcements	Number of reinforcement received by the animal (priming excluded)	
Nb. Detections	Total number of animal detections in the food holder	Not Available
Nb. Correct	Number of entries into the “Correct” state	
Nb. Incorrect	Number of entries into the “Incorrect” state	
Nb. Omission	Number of entries into the “Omission” state	
Nb. Premature	Number of entries into the “Premature” state	
Nb. Perseverative	Number of entries into the “Perseverative” state	
Nb. Time-out Resp.	Number of nose poke detected during the Time-out period. Calculated making the sum of the entries into the “Time-out hole” and “Time-out hole premature”.	
Choice accuracy	% of Correct responses = (Nb Correct / (Nb Correct + Nb Incorrect))x100	
% omissions	% of Omission = (Nb Omission / (Nb Correct + Nb Incorrect + Nb Omission))x100	
Mean correct response latency	Mean duration of time spent between the initiation of the “Hole n” states and the related “Correct” state of the same trial.	
Mean incorrect response latency	Mean duration of time spent between the initiation of the “Hole n” states and the related “Incorrect” state of the same trial.	
Mean magazine latency	Mean duration of time spent between the initiation of the “reinforcement” state and the “ITI” when the “Wait animal detection in food holder to allow next reinforcement” is checked. If the option is not checked, the case is not filled.	Not Available
Nb. Resp. Total	Number of nose pole into the Holes	
Nb. Resp. H1	Number of nose poke detected in the Hole 1	
Nb. Resp. H2	Number of nose poke detected in the Hole 2	
Nb. Resp. H3	Number of nose poke detected in the Hole 3	
Nb. Resp. H4	Number of nose poke detected in the Hole 4	
Nb. Resp. H5	Number of nose poke detected in the Hole 5	
Nb. Resp. H6	Number of nose poke detected in the Hole 6	
Nb. Resp. H7	Number of nose poke detected in the Hole 7	
Nb. Resp. H8	Number of nose poke detected in the Hole 8	
Nb. Resp. H9	Number of nose poke detected in the Hole 9	
Nb. Premature H1	Number of nose poke detected in the Hole 1 during the ITI	
Nb. Premature H2	Number of nose poke detected in the Hole 2 during the ITI	
Nb. Premature H3	Number of nose poke detected in the Hole 3 during the ITI	
Nb. Premature H4	Number of nose poke detected in the Hole 4 during the ITI	

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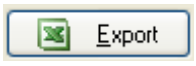
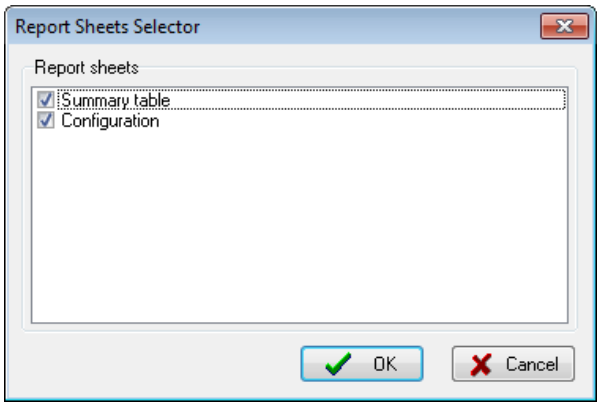
Column	Nine Hole Box type	Nine Hole Nine Pellets Box type
Nb. Premature H5	Number of nose poke detected in the Hole 5 during the ITI	
Nb. Premature H6	Number of nose poke detected in the Hole6 during the ITI	
Nb. Premature H7	Number of nose poke detected in the Hole 7 during the ITI	
Nb. Premature H8	Number of nose poke detected in the Hole8 during the ITI	
Nb. Premature H9	Number of nose poke detected in the Hole 9 during the ITI	
Nb. Correct H1	Number of Correct response made into the Hole 1	
Nb. Correct H2	Number of Correct response made into the Hole 2	
Nb. Correct H3	Number of Correct response made into the Hole 3	
Nb. Correct H4	Number of Correct response made into the Hole 4	
Nb. Correct H5	Number of Correct response made into the Hole 5	
Nb. Correct H6	Number of Correct response made into the Hole 6	
Nb. Correct H7	Number of Correct response made into the Hole 7	
Nb. Correct H8	Number of Correct response made into the Hole 8	
Nb. Correct H9	Number of Correct response made into the Hole 9	
Nb. Incorrect H1	Number of Incorrect response made into the Hole 1	
Nb. Incorrect H2	Number of Incorrect response made into the Hole 2	
Nb. Incorrect H3	Number of Incorrect response made into the Hole 3	
Nb. Incorrect H4	Number of Incorrect response made into the Hole 4	
Nb. Incorrect H5	Number of Incorrect response made into the Hole 5	
Nb. Incorrect H6	Number of Incorrect response made into the Hole 6	
Nb. Incorrect H7	Number of Incorrect response made into the Hole 7	
Nb. Incorrect H8	Number of Incorrect response made into the Hole 8	
Nb. Incorrect H9	Number of Incorrect response made into the Hole 9	
Nb. Time-out Resp. H1	Number of nose poke detected in the Hole 1 during the Time-out	
Nb. Time-out Resp. H2	Number of nose poke detected in the Hole 2 during the Time	
Nb. Time-out Resp. H3	Number of nose poke detected in the Hole 3 during the Time	
Nb. Time-out Resp. H4	Number of nose poke detected in the Hole 4 during the Time	
Nb. Time-out Resp. H5	Number of nose poke detected in the Hole 5 during the Time	
Nb. Time-out Resp. H6	Number of nose poke detected in the Hole 6 during the Time	
Nb. Time-out Resp. H7	Number of nose poke detected in the Hole 7 during the Time	
Nb. Time-out Resp. H8	Number of nose poke detected in the Hole 8 during the Time	
Nb. Time-out Resp. H9	Number of nose poke detected in the Hole 9 during the Time	

9.5.2.3 Data Exportation



Press the **Export** button to export the Numerical tables directly in Excel format. The exported tables can be filtered using the Report Sheets Selector panel.



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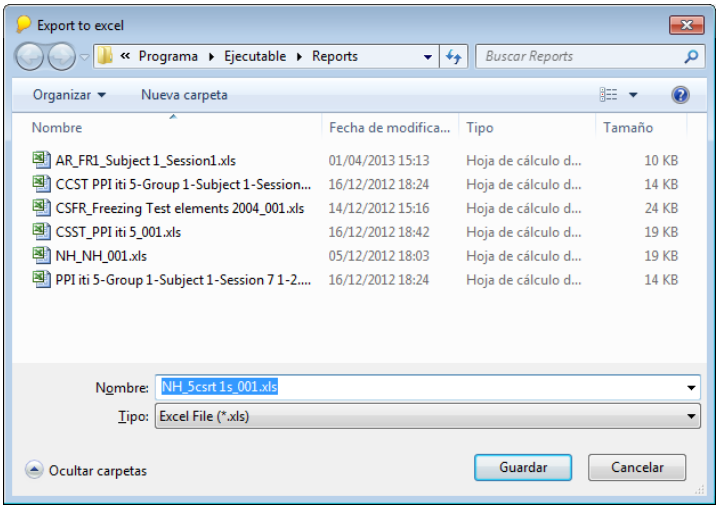
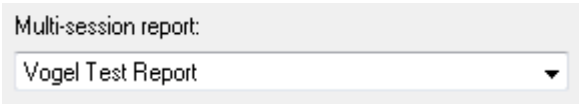
The Excel file name and location can also be defined by the user in the **Export to excel** saving panel.



9.5.3 PACKWIN Operant Vogel Test Module (VT)

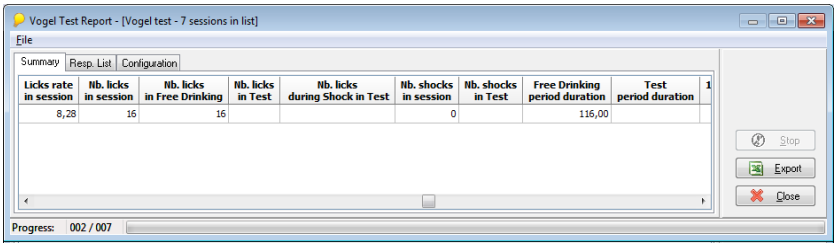


PACKWIN provides a specific Vogel Test report for each Vogel test assistant (Vogel Pre-Test or Vogel Test procedures).

Reports are predefined so no Subject & Session, Calculation, or Time selection configuration is available.

Press the **Analyse** button to preview the data tables before their exportation to Excel.

The data are presented into 3 different tags: Summary table, Resp. List and Configuration (see tables bellow for the detail of the content of each report).



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9.5.3.1 Vogel PreTest Report - Summary

Column	Description
Subject group	Name of the group associated to the subject (user-defined)
Subject name	Name of the subject (user-defined)
Treatment	Treatment (user-defined)
Gender	Animal Gender (user-defined)
Species	Animal species (user-defined)
Subject code	Subject code (internal number)
Group code	Group code (internal number)
Protocol	Protocol name (user-defined)
Session number	Number of the corresponding session
Experiment duration	Time spent between the START and END state
1st lick latency in session	Time latency of the first lick detected after the beginning of the session (START state). When no licks are detected, the case remains empty. (in seconds)
Drinking time in session	Total time the subject spent drinking/licking during the whole session. Technically, it is the total duration in which the input element "Lick" has been in the "Detected" status (in seconds).
Licks rate in session	Lick rate during the whole session. Technically, it is the ratio between the total number of licks and the total duration of the session (in licks/min).
Nb. licks in session	Total number of licks detected during the whole session.

9.5.3.2 Vogel PreTest Report – Resp. List

Column	Description
Subject group	Name of the group associated to the subject (user-defined)
Subject name	Name of the subject (user-defined)
Treatment	Treatment (user-defined)
Gender	Animal Gender (user-defined)
Species	Animal species (user-defined)
Subject code	Subject code (internal number)
Group code	Group code (internal number)
Protocol	Protocol name (user-defined)
Session number	Number of the corresponding session
Index	Index number of the response
Element/Status	Name of the activated input element and status related to the current response
Time	Starting time of the response(in seconds)
Duration	Duration of the response (in seconds)
START State	Name of the State in which the current response has started
END State	Name of the State in which the current response has ended

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9.5.3.3 Vogel PreTest Report – Configuration

Column	Description
Subject group	Name of the group associated to the subject (user-defined)
Subject name	Name of the subject (user-defined)
Treatment	Treatment (user-defined)
Gender	Animal Gender (user-defined)
Species	Animal species (user-defined)
Subject code	Subject code (internal number)
Group code	Group code (internal number)
Protocol	Protocol name (user-defined)
Session number	Number of the corresponding session
Box type	Type of Box associated with the protocol
Yoked procedure	Status of the yoked procedure: Independent/Master of Box (...)/Slave of Box (...)
Session date	Date and clock time in which the session started
Remarks	Text entered in the Comments section of the Saving panels
Module	Name of the experimental module used for configuring the protocol
Test	Name of the Assistant used for configuring the protocol
Lickometer element	Name of the element of the cage selected as Lickometer. When using the Panlab Vogel cage, the element is pre-selected as Lick
Shocker element	Name of the element of the cage selected as Shocker. When using the Panlab Vogel cage, the element is pre-selected as Shock
House light element	Name of the element of the cage selected as House Light. When using the Panlab Vogel cage, this case remains empty
House light	House light status: ON/OFF. When using the Panlab Vogel cage, this case remains empty
Stop Pre-Test after	Condition for ending the experiment: (...) min elapsed, After (...) licks, (...) min without response. When no option is selected, this case remails empty. The user can always stop the session manually.

9.5.3.4 Vogel Test Report - Summary

Column	Description
Subject group	Name of the group associated to the subject (user-defined)
Subject name	Name of the subject (user-defined)
Treatment	Treatment (user-defined)
Gender	Animal Gender (user-defined)
Species	Animal species (user-defined)
Subject code	Subject code (internal number)
Group code	Group code (internal number)
Protocol	Protocol name (user-defined)
Session number	Number of the corresponding session
Experiment duration	Total duration of the experiment (in seconds). Technically, it is the time spent between the START and END state.
1st lick latency in session	Time latency of the first lick detected after the beginning of the session (START state; in seconds). When no licks are detected, the case remains empty.
1st lick latency in test	Time latency of the first lick detected after the beginning of the TEST period (Test INI state; in seconds). When no licks are detected, the case remains empty.
Drinking time in session	Total time the subject spent drinking/licking during the whole session (in seconds). Technically, it is the total duration in which the input element “Lick” has been in the “Detected” status.

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Column	Description
Drinking time in Free Drinking	Total time the subject spent drinking/licking during the Free Drinking period. When the protocol doesn't contain any Free Drinking period, this case remains empty. When a lick begins in the Free Drinking period and ends in the Test period, the duration of this lick is added to this calculation.
Drinking time in Test	Total time the subject spent drinking/licking during the Test period (only for the licks that have been initiated during the Test period.
Licks rate in session	Lick rate during the whole session. Technically, it is the ratio between the total number of licks and the total duration of the session (in licks/min).
Nb. licks in session	Total number of licks detected during the whole session.
Nb. licks in Free Drinking	Total number of licks initiated in the Free Drinking period. When the protocol doesn't contain any Free Drinking period, this case remains empty.
Nb. licks in Test	Total number of licks initiated in the Test period.
Nb. licks during Shock in Test	Total number of licks detected during the shocks delivered in the Test period.
Nb. shocks in session	Total number of shocks delivered during the whole session.
Nb. shocks in Test	Total number of shocks delivered during the Test period.
Free Drinking period duration	Duration of the Free Drinking period (in seconds). When the protocol doesn't contain any Free Drinking period, this case remains empty.
Test period duration	Duration of the Test period (in seconds)
1st shock latency in session	Time latency of the first shock detected after the beginning of the session (START state; in seconds). When no shock is detected, the case remains empty.
1st shock latency in test	Time latency of the first shock detected after the beginning of the Test period (Test INI state; in seconds). Only given when the "After the first shock" option has been chosen for beginning the Test period.
Mean time for completing block of licks	Mean time between the first lick of the block of licks configured in the shock distribution mode ("every (...) licks") and the resultant shock (in seconds)
Suppression time in Test	Time latency between the first shock of the Test period and first following lick (in seconds)
Mean Inter-response time in Test	Mean Inter-licks interval time during the Test period (in seconds)
Mean shock latency in Test	Mean inter-shock interval time during the test (including the latency between the beginning of the Test period and the first shock).
Licks rate in Free Drinking	Lick rate during the Free Drinking period. Technically, it is the ratio between the total number of licks during the Free Drink period and the total duration of the Free Drinking period (in licks/min). When the protocol doesn't contain any Free Drinking period, this case remains empty.
Licks rate in Test	Lick rate during the Test period. Technically, it is the ratio between the total number of licks during the Test period and the total duration of the Test period (in licks/min).

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When the "after first lick" option is chosen as a condition for beginning the Test, this first lick is not included in the following calculations:

- Drinking time in Test
- Nb. licks in Test
- Mean lick latency in test
- Mean Inter-response time in Test
- Time for completing block of licks
- Licks rate in Test

When the "after first shock" option is chosen as a condition for beginning the Test, this first lick is not included in the following calculations:

- Nb. shocks in Test
- 1st shock latency in test
- Mean shock latency in Test

9.5.3.5 Vogel Test Report – Resp. List

The Resp. List sheet of the Vogel Test Report contains exactly the same information that the Resp. List of the Vogel PreTest Report.

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9.5.3.6 Vogel Test Report – Configuration

Column	Description
Subject group	Name of the group associated to the subject (user-defined)
Subject name	Name of the subject (user-defined)
Treatment	Treatment (user-defined)
Gender	Animal Gender (user-defined)
Species	Animal species (user-defined)
Subject code	Subject code (internal number)
Group code	Group code (internal number)
Protocol	Protocol name (user-defined)
Session number	Number of the corresponding session
Box type	Type of Box associated with the protocol
Yoked procedure	Status of the yoked procedure: Independent/Master of Box (...)/Slave of Box (...)
Session date	Date and clock time in which the session started
Module	Name of the experimental module used for configuring the protocol
Test	Name of the Assistant used for configuring the protocol
Lickometer element	Name of the element of the cage selected as Lickometer. When using the Panlab Vogel cage, the element is pre-selected as Lick
Shocker element	Name of the element of the cage selected as Shocker. When using the Panlab Vogel cage, the element is pre-selected as Shock
House light element	Name of the element of the cage selected as House Light. When using the Panlab Vogel cage, this case remains empty
House light	House light status: ON/OFF. When using the Panlab Vogel cage, this case remains empty
Free Drinking	Indicates whether the user has activated (“Active”) or not (“Inactive”) the Free Drinking option
End free drinking	End after (…) minutes/ End after (…) licks. The case remains empty when the Free Drinking option is unchecked.
Test START condition	Immediately/After the first Lick/After the first shock
Stop session if no Test Start after	(...) minutes. The case remains empty when this option is unchecked
Shock duration	(…) milliseconds
Shock distribution mode	Every (...) licks
Stop started test after	(…) minutes

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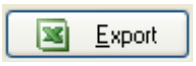
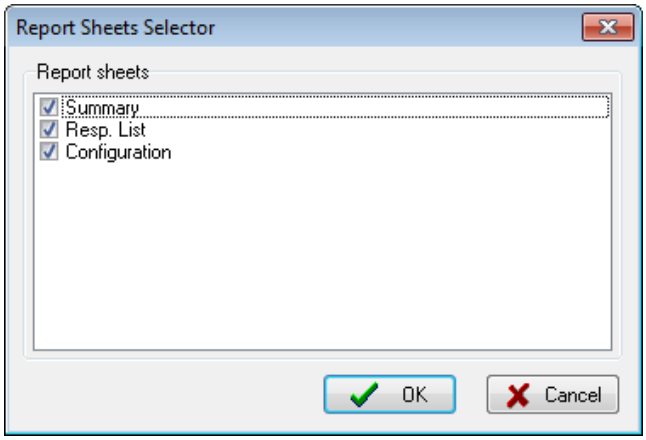
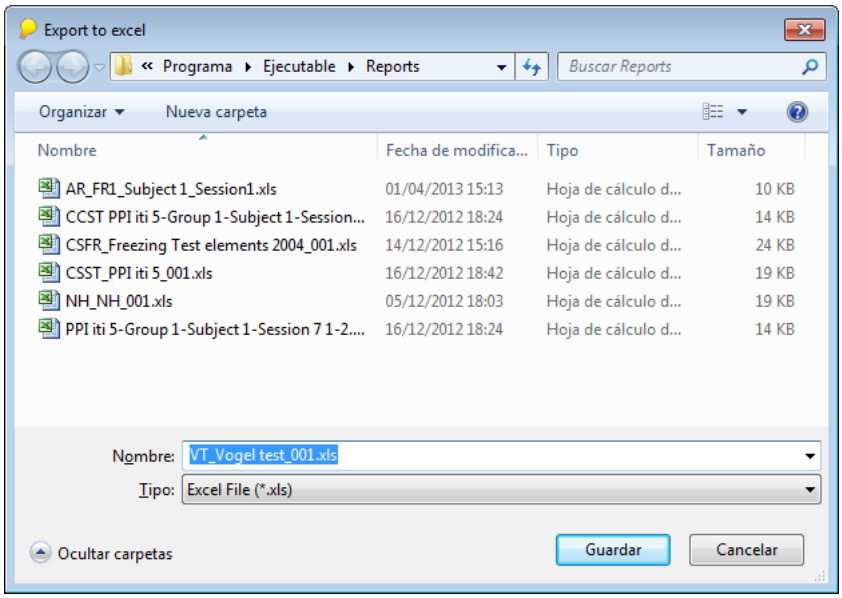
9.5.3.7 Data Exportation



Press the **Export** button to export the Numerical tables directly in Excel format. The exported tables can be filtered using the Report Sheets Selector panel.



The Excel file name and location can also be defined by the user in the **Export to excel** saving panel.



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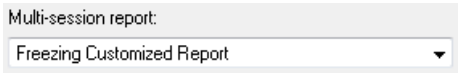
9.5.4 PACKWIN Freezing Module (CSFR)

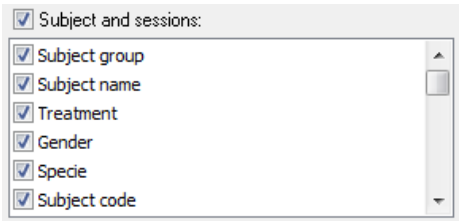
The FREEZING module provides a single "Freezing Customized Report" for batch analysis.



9.5.4.1 Data selection

SELECT SUBJECT AND SESSIONS DATA

- Select the **Subject and Session** information to be included in the report.



- To select an item, check the associated box ☑.
- To unselect an item, uncheck the associated box ☐.
- To select or unselect all the items, check or uncheck the **Subject and sessions** title box.

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The available options are:

Subject group	Name of the group associated to the subject (user-defined)
Subject name	Name of the subject (user-defined)
Treatment	Treatment (user-defined)
Gender	Animal Gender (user-defined)
Species	Animal species (user-defined)
Subject code	Subject code (internal number)
Group code	Group code (internal number)
Date	Date of session registering
Clock time	Time of session registering
Experiment file	Name of the experiment file containing the session
Remarks	Remarks (user-defined)
Box type	Box type associated to the protocol used in the registered session
Box number	Number of the box in which the session has been registered
Protocol	Protocol used in the registered session
Session number	Number of session for the animal used in the session
Session duration	Full duration of the registered session
Start interval	Start time set for analysis
End interval	End time set for analysis
Split time	Split time set for analysis
Sample rate	Sampling frequency = number of samples/second registered during the data acquisition process (see chapter 8.3.2.3.2.).
Channel Gain	Gain set in the software before starting the data acquisition session (see chapter 8.3.2.3.1).
Threshold up	Low activity Threshold set in the graph
Threshold down	High activity Threshold set in the graph
Min. Freezing Duration	The minimum freezing duration (sec) to detect freezing episodes configured for this analysis report
Breathing filter	Enabling status (true/false) of the breathing filter configured for this analysis report.

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SELECT THE CALCULATIONS TO BE REPORTED IN THE REPORT

The available calculations are:

- To select a calculation, check the associated box ☑.
- To unselect a calculation, uncheck the associated box ☐.
- To select or unselect all the calculations, check or uncheck the **Calculations** title box.

The available calculations are:

Calculation	**Meaning**
Freezing Duration	Total accumulated duration (sec) of freezing episodes. States can be filtered using the S filter.
Activity Duration	Total accumulated duration (sec) of activity episodes. States can be filtered using the S filter.
% Freezing	Percentage of freezing time (%) in the session analysed. States can be filtered using the S filter.
% Activity	Percentage of activity time (%) in the session analysed. States can be filtered using the S filter.
Nr. Freezing Episodes	Total number of freezing episodes starting in the interval of time analysed. States can be filtered using the S filter.
Episodes List	Number and Duration of the Freezing, Low and High activity events as selected using the E filter and calculated for each user-defined interval of time.

The state of the protocols to which the calculation will be applied, and Events selected in the Episodes List can be filtered using the following filters:

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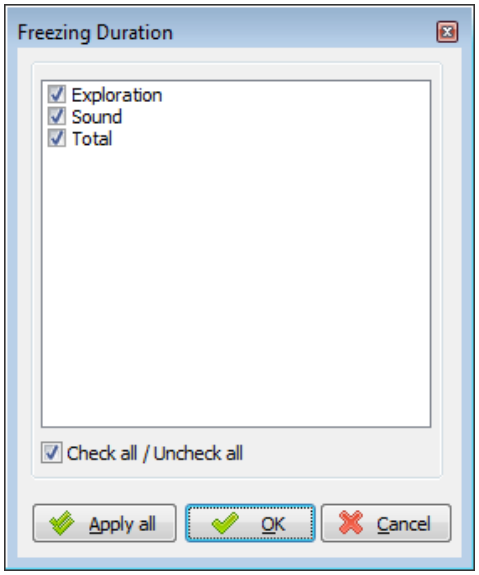
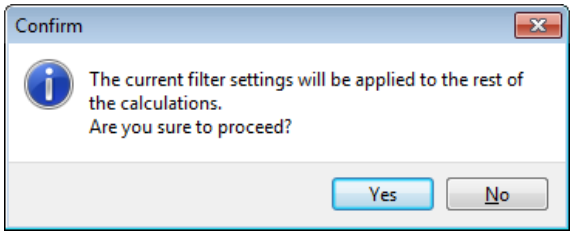
Apply a state filter

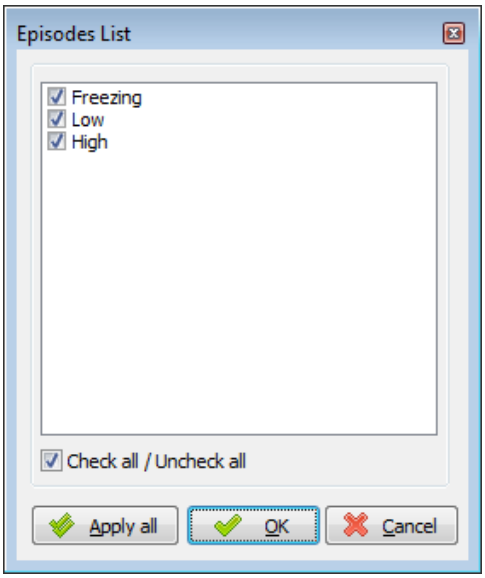


- To select a state, check the associated box ☑.
- To unselect a state, uncheck the associated box ☐.
- More than one state can be chosen.

Use the **Apply all** button for applying the configuration to all the calculations using the State filter.



Apply an Event filter



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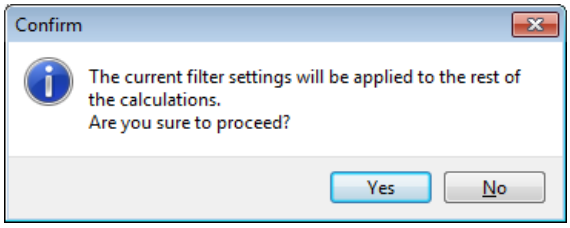
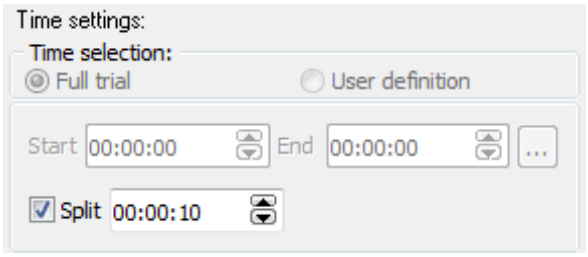
- To select an Event, check the associated box ☑.
- To unselect an Event, uncheck the associated box ☐.

Use the **Apply all** button for applying the configuration to all the calculations using the Element filter.



9.5.4.2 Time selection

FREEZING sessions will be always analysed through their whole duration so the "Time selection" section will be always disabled.



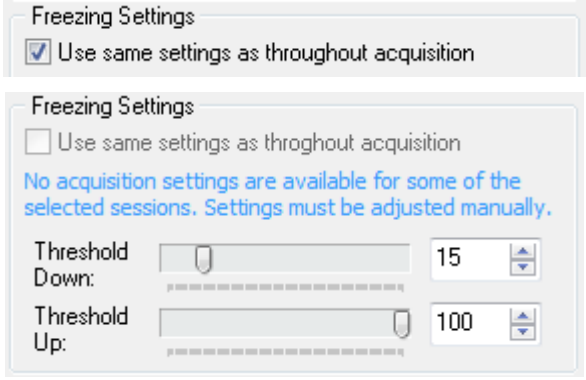
A split option is available for the exportation of the Episode table summarizing the amount of activity episode calculated for each user-defined subinterval of time.

9.5.4.3 FREEZING analysis settings

The **Freezing Settings** section of the analysis window allows configuration of the signal thresholds that will be used during the analysis process.

Check the "Use same settings as throughout acquisition" box for using the same threshold settings used during the acquisition process.

Uncheck the box to set new thresholds and apply them to the selected sessions.



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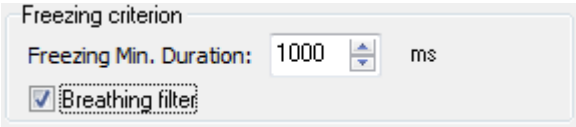
If the "Use same settings as throughout acquisition" box is checked and any of the sessions selected in the table were acquired with older versions of the FREEZING software, the signal thresholds used during acquisition may not be available. In that case, the Threshold Down and Threshold Up set here above will be taken into account for these old sessions.

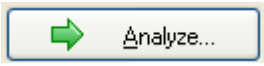
If the "Use same settings as throughout acquisition" box is unchecked, then all the sessions (old and new) will use the Threshold Down and Threshold Up set here above.

The value of the Threshold Up cannot be ≤ to the value of the Threshold Down.

The **Freezing criterion** section allows configuring the duration filter and enabling/disabling the breathing filter (see o 8.3.2.3.3. for details).

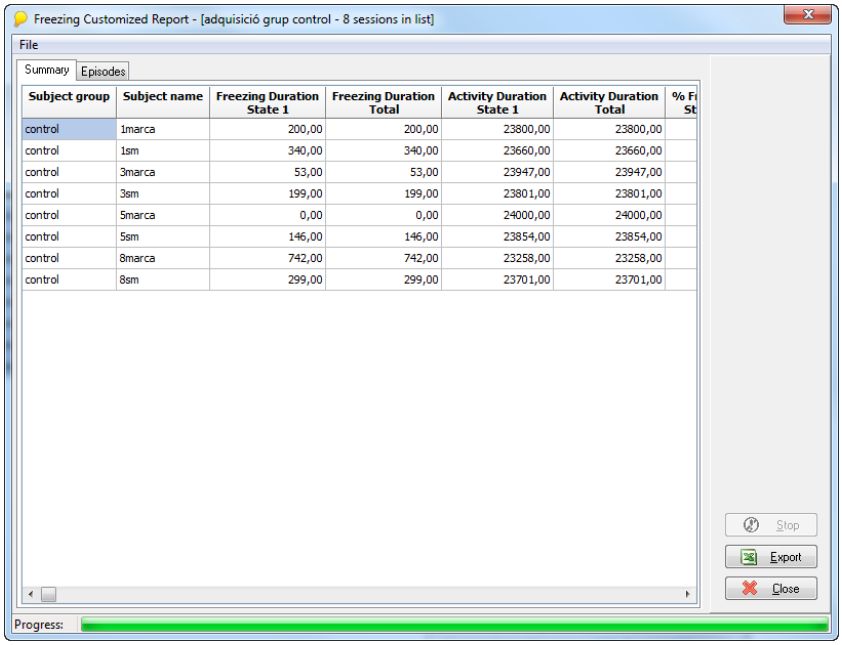


9.5.4.4 Data report preview



Press the **Analyse...** button to preview the data reports.

The **Summary** tab contains all the Subject & Session items and calculations previously selected. The report displays 1 line for each subject.



Subject group	Subject name	Freezing Duration State 1	Freezing Duration Total	Activity Duration State 1	Activity Duration Total	% Fi St
control	1marca	200,00	200,00	23800,00	23800,00	
control	1sm	340,00	340,00	23660,00	23660,00	
control	3marca	53,00	53,00	23947,00	23947,00	
control	3sm	199,00	199,00	23801,00	23801,00	
control	5marca	0,00	0,00	24000,00	24000,00	
control	5sm	146,00	146,00	23854,00	23854,00	
control	8marca	742,00	742,00	23258,00	23258,00	
control	8sm	299,00	299,00	23701,00	23701,00	

The **Episodes** tab contains the data of the same Episode table as the one displayed in the Single session analysis but presented with 1 line for each subject. The **Episodes** tab contains the information associated to the freezing events

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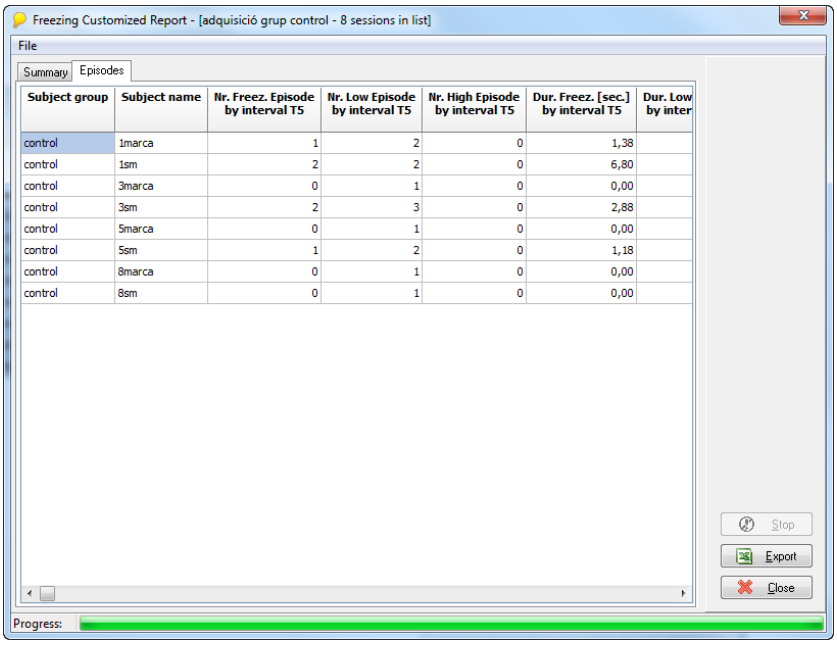
calculated per each time interval (as configured in the **Split** option described in chapter 9.5.4.2.).

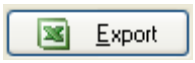
The following image shows an example of analysis of multiple sessions with the **Split** option configured to 5 sec.



Subject group	Subject name	Nr. Freez. Episode by interval T5	Nr. Low Episode by interval T5	Nr. High Episode by interval T5	Dur. Freez. [sec.] by interval T5	Dur. Low by inter
control	1marca	1	2	0	1,38	
control	1sm	2	2	0	6,80	
control	3marca	0	1	0	0,00	
control	3sm	2	3	0	2,88	
control	5marca	0	1	0	0,00	
control	5sm	1	2	0	1,18	
control	8marca	0	1	0	0,00	
control	8sm	0	1	0	0,00	

The title of each column is composed by the name of the calculation and the end time (in sec) of the interval represented (i.e. "T5" represents the interval from 0,00 to 4,99 sec, "T10" represents the interval from 5,00 to 9,99 sec and so on).

9.5.4.5 Data exportation



Press the **Export** button for exporting the tables directly in Excel format.

The Excel file name and location can also be defined in the **Export to excel** saving panel.

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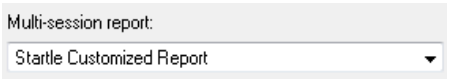
9.5.5 PACKWIN Startle Module (CSST)

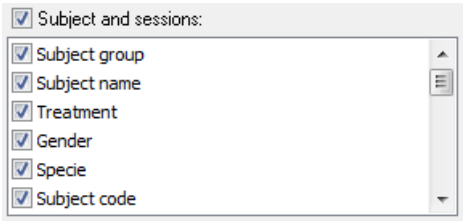
The STARTLE module provides a single "Startle Customized Report" for batch analysis.



9.5.5.1 Data selection

SELECT SUBJECT AND SESSIONS DATA

- Select the **Subject and Session** information to be included in the report.



- To select an item, check the associated box ☑.
- To unselect an item, uncheck the associated box ☐.
- To select or unselect all the items, check or uncheck the **Subject and sessions** title box.

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The available options are:

Subject group	Name of the group associated to the subject (user-defined)
Subject name	Name of the subject (user-defined)
Treatment	Treatment (user-defined)
Gender	Animal Gender (user-defined)
Species	Animal species (user-defined)
Subject code	Subject code (internal number)
Group code	Group code (internal number)
Date	Date of session registering
Clock time	Time of session registering
Experiment file	Name of the experiment file containing the session
Remarks	Remarks (user-defined)
Box type	Box type associated to the protocol used in the registered session
Box number	Number of the box in which the session has be registered
Protocol	Protocol used in the registered session
Session number	Number of session for the animal used in the session
Session duration	Full duration of the registered session
Start interval	Start time set for analysis
End interval	End time set for analysis
Split time	Split time set for analysis
Sample rate	Sampling frequency = number of samples/second registered during the data acquisition process (see chapter 8.3.2.3.2.).
Channel Gain	Gain set in the software before starting the data acquisition session (see chapter 8.3.2.3.1).
Threshold up	Threshold used for defining the END of the startle reflex peak.
Threshold down	Threshold used for defining the START of the startle reflex peak.

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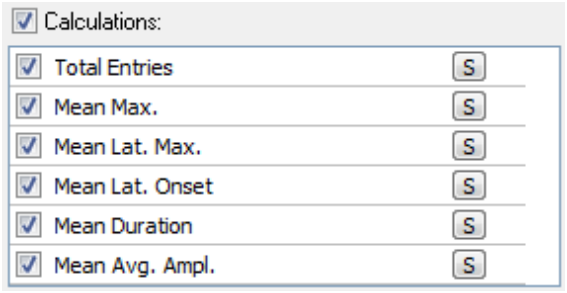
SELECT THE CALCULATIONS TO BE REPORTED IN THE REPORT

The available calculations are:



- To select a calculation, check the associated box ☑.
- To unselect a calculation, uncheck the associated box ☐.
- To select or unselect all the calculations, check or uncheck the **Calculations** title box.

The available calculations are:

Calculation	Meaning
Total entries	Total number of entries into the state. States can be filtered using the S filter.
Mean Max.	Average max amplitude (%) of the startle responses. States can be filtered using the S filter.
Mean Lat. Max.	Average latency (msec) to reach the max amplitude of the startle responses. States can be filtered using the S filter.
Mean Lat. Onset	Average latency (msec) to reach the onset status in the startle responses. States can be filtered using the S filter.
Mean Duration	Average duration (msec) of the startle response episodes. States can be filtered using the S filter.
Mean Area	Average signal amplitude (no units). States can be filtered using the S filter.

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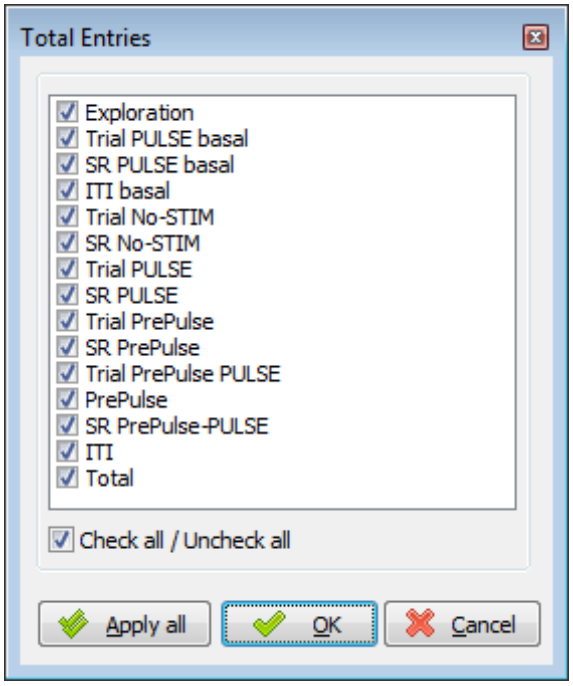
The state of the protocols to which the calculation will be applied can be filtered using the following filter:

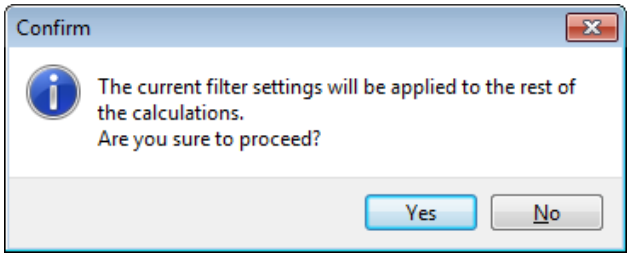
5

Apply a state filter



- To select a state, check the associated box ☑.
- To unselect a state, uncheck the associated box ☐.
- More than one state can be chosen.

Use the **Apply all** button for applying the configuration to all the calculations using the State filter.



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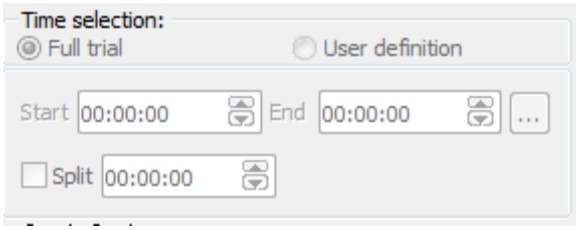
9.5.5.2 Time selection

STARTLE sessions will be always analysed through their whole duration so the "Time selection" section will be always disabled. Moreover, it is not possible to analyse data in a user-defined subinterval of time, so Split is also always disabled.

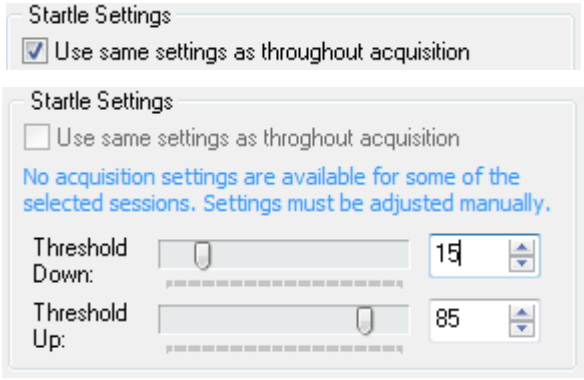


9.5.5.3 STARTLE analysis settings

The **Startle Settings** section of the analysis window allows for configuring the signal thresholds that will be used during the analysis process.

Check the "Use same settings as throughout acquisition" box to use the same threshold settings used during the acquisition process.

Uncheck the box for setting new thresholds and applying them to the selected sessions.



If the "Use same settings as throughout acquisition" box is checked and any of the sessions selected in the table were acquired with older versions of the STARTLE software, the signal thresholds used during acquisition may not be available. In that case, the Threshold Down and Threshold Up set here above will be taken into account for these old sessions.

If the "Use same settings as throughout acquisition" box is unchecked, then all the sessions (old and new) will use the Threshold Down and Threshold Up set here above.

The value of the Threshold Up cannot be ≤ to the value of the Threshold Down.

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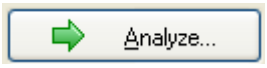
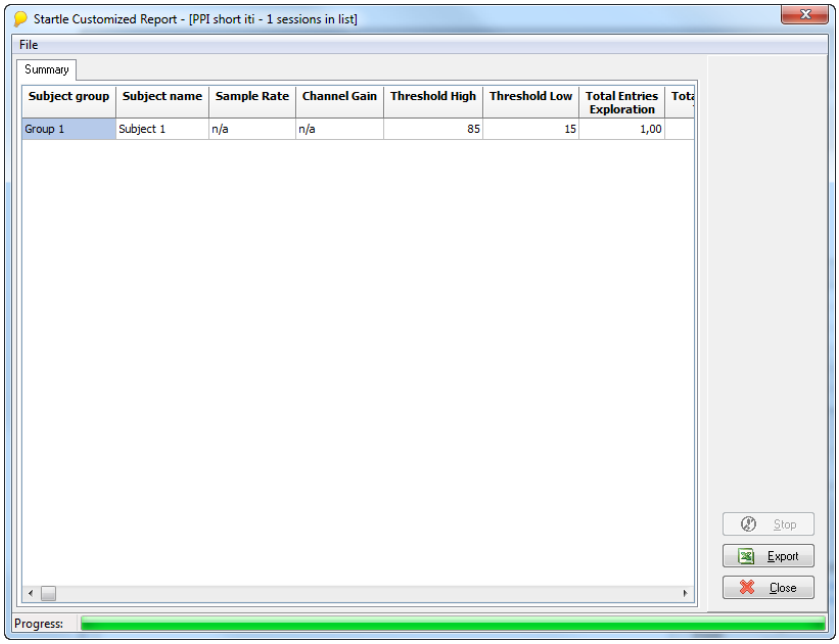


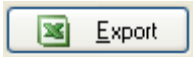
9.5.5.4 Data report preview

Press the **Analyse...** button to preview the data reports.

A single **Summary** tab is shown containing all the Subject & Session items and calculations previously selected. The report displays 1 line for each subject.



9.5.5.5 Data exportation



Press the **Export** button for exporting the tables directly in Excel format.

The Excel file name and location can also be defined in the **Export to excel** saving panel.

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10 Experimental files management

10.1 Structure of the experimental file

The experimental file created with FREEZING and STARTLE is divided into four different files sharing exactly the same name (user-defined) but associated with different extension:

- EXP extension: contains all the information related to the subjects and protocols
- INI extension: contains all the default and current settings
- SSN extension: contains all the information about the registered session (date, hour, associated subject information, associated protocol…)
- RAW extension: contains all the data/calculation registered in each session

An inappropriate operation applied by the user on these files may make them unusable for experimental use. In order to avoid such problems, the next recommendations have to be followed:

- Never try to directly open the files. The EXP file can only be opened through the FREEZING or STARTLE application (File/Open)
- The 4 files of the same experiment need to ALWAYS remain in the same folder and cannot be separated into different folders.
- The 4 files of the same experiment always need to share the same name.
- An experimental file created with the STARTLE application cannot be opened, used, or analysed using the FREEZING application (and vice versa)

10.2 Changing the localization

The location of the experimental file can be changed by the user. To do this, it is absolutely mandatory to move/copy all the 4 files associated (*.exp, *.ini, *.ssn" and *.raw) together to the new location.

10.3 Changing file name

The name of the experimental file can be changed by the user after their creation. To do this, it is absolutely mandatory to change the name in the 4 files associated (*.exp, *.ini, *.ssn" and *.raw). Of course, the new name has to be the same in the 4 files.

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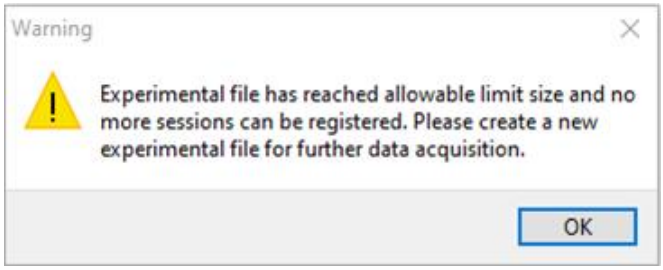
10.4 Duplicating an experimental file

The copy function of the Windows operating system can be used to duplicate an experimental file. To do this, it is absolutely mandatory to duplicate the 4 files associated (*.exp, *.ini, *.ssn" and *.raw).

10.5 Size limitations

The number of sessions that can be registered into the experimental file is unlimited. Nevertheless, the optimal use of the file for protocol configuration, acquisition, and **analysis is only guaranteed until the size of 1 Gb for the *.raw file.** It is then highly recommended to create a new experimental file each time a new experiment is began.

When the user tries to open an Experimental file with a size > than 1 Gb, the following message is displayed.



If the user aims to use the same protocols in different experiments, we recommend keeping an original copy of the experimental file filled with the protocols but empty of registered sessions. For each new experiment, the user can make a copy of the original file and then execute again the protocols contained in it for registering a new set of sessions.

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11 PACKWINCS Extension

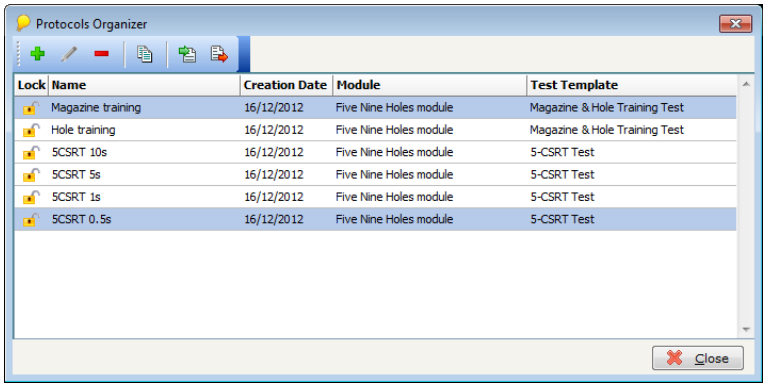
The combination of a PACKWIN experiment module (PACKWIN HO or PACKWIN VT) with the PACKWIN-CS module provides several advantages for those user who need an optimal flexibility in the protocols and data reports configuration.

11.1 Combining Assistant tools and the State editor tool

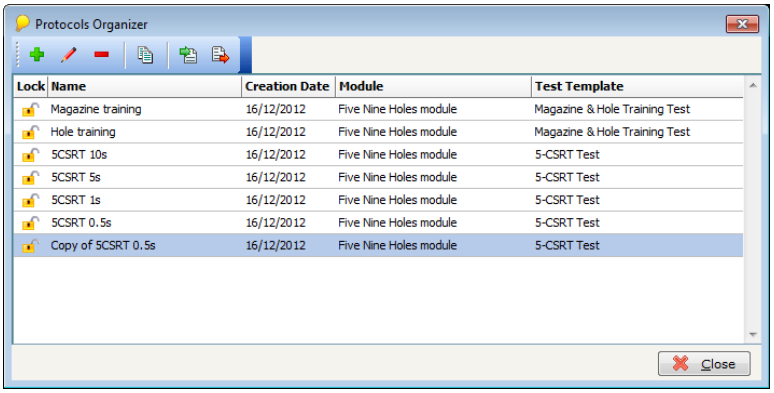
The PACKWIN-HO and PACKWIN-VT protocol assistants can be used as a time saving way to configure a standard protocol and can be opened afterwards with the STATE EDITOR tool if the user would like to modify any of the characteristics of the protocol (protocol structure. number of states and link between them, State names etc...).

Here is a recommended way to do that:

- Open a 5/9 hole or Vogel test experiment created with the PACKWIN-HO or PACKWIN-VT module.
- Open the protocol organizer showing all the protocols created with the PACKWIN-HO or PACKWIN-VT module assistants and choose the protocol to be modified.



- Do a copy of the protocol:



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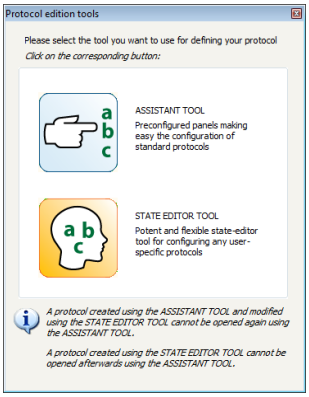
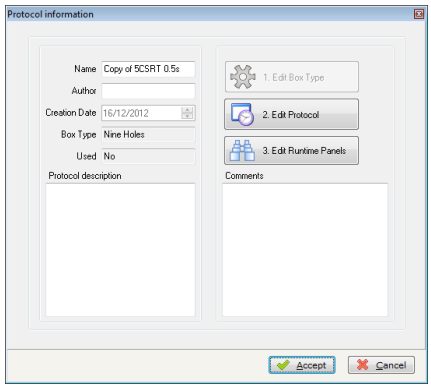
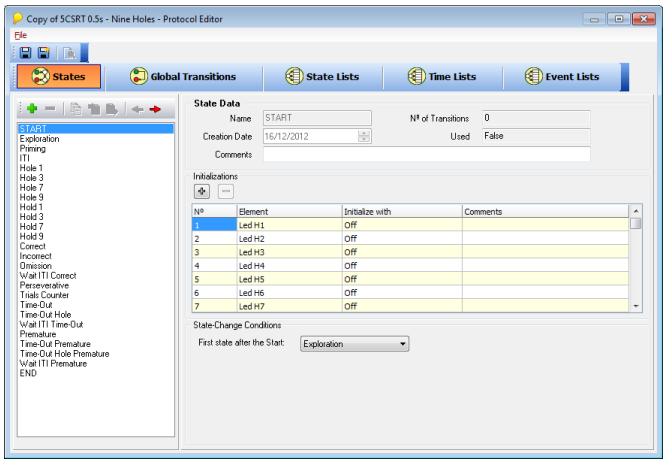
- Double-click on the protocol and choose the STATE EDITOR TOOL option (instead of the ASSISTANT TOOL option).





- Modify any of the steps of the protocol as required (see chapter 7.2. for detailed information of how to edit a protocol using the STATE EDITOR TOOL.



11.2 Editing the Run-Time panel

The PACKWIN-CS module provides the possibility to change/edit the information appearing in both the State and Transition tables shown in the data acquisition Run-Time panel.

See chapter 7.2.7. for details about the how to edit a Run-Time panel with the PACKWIN-CS module.

11.3 Editing a customized report

The PACKWIN-CS module provides the possibility to choose the calculations to be shown in the data reports.

See chapter 9.5.1. for details about the how to edit a data report with the PACKWIN-CS module.

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12 Record-it! Media Integration

PACKWIN can now be integrated with RECORD-IT! Media for synchronized video recording during data acquisition.

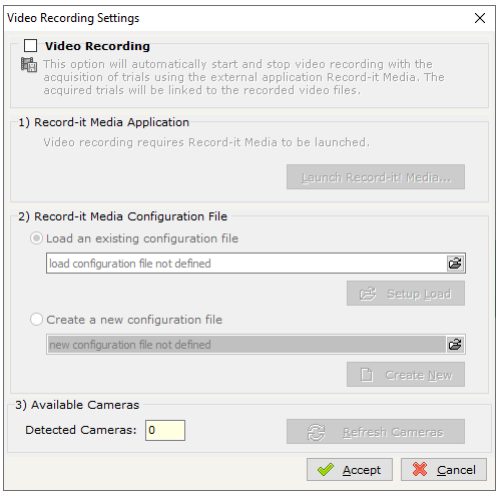
12.1 Record-it! Media Installation

To integrate RECORD-IT! Media with PACKWIN, RECORD-IT! Media must be installed on the same computer as PACKWIN.

Check the Record-It! Media manual for the detailed instructions on how to install the program and the video cameras.

12.2 Video Recording Settings

To configure cameras for video recording, select "Configuration" and then "Video Recording Settings". The following panel will appear:



Video recording is disabled by default. To activate it, check the "Video Recording" box, then follow the three steps described in the panel:

1) Launch the RECORD-IT! Media by pressing the "Launch Record-it! Media" button. If the RECORD-IT! Media program is already running, this button will be disabled.

2) Select a camera configuration file:

   a. If you already have a camera configuration file saved, select the "Load an existing configuration file" option, using the navigation button and press the "Setup Load" button, to load this file into Record-it! Media.

   b. If you don't have a configuration file yet, select the option "Create a new configuration file", choose the name of the

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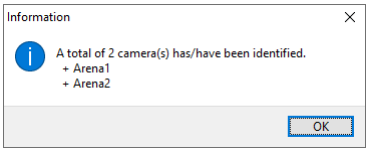
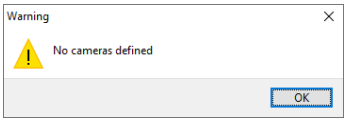
configuration file and press the "Create New" button, to create this file in Record-it! Media.

The configuration file will be loaded into RECORD-IT! Media. You can then review the camera settings, add or remove cameras, and rename them.

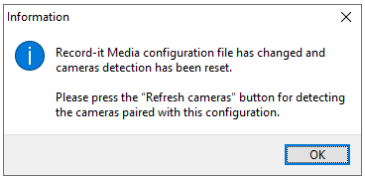
3) Press the "Refresh Cameras" button, so that PACKWIN retrieves the list of cameras finally configured.



If the setup file does not contain a camera definition, the following error message will be displayed:



If the cameras have been configured correctly, click the Accept button to close the form. If the configuration is wrong or incomplete the following error message will be displayed.



This setting is saved at the experimental file level, i.e. the next time you load a certain experimental file, the "Video Recording Settings" information will be retrieved. It is necessary to refresh the camera list to ensure that all the cameras defined are available for recording.

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13 Contact Information

We are available to help you with your questions and concerns. Should you hit a roadblock or need some additional training, please feel free to visit the HBIO Behavioral Support Center at https://support.behavior.hbiosci.com to find articles and helpful information in our knowledge base or submit a ticket. We are happy to help!

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