USER MANUAL

SHUTAVOID 1.9.01

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1. INTRODUCTION

The SHUTAVOID for Windows is designed by the Software Department of Panlab, to control experiments carried out simultaneously with up of up to 8 shuttle boxes. The program controls the presentation of visual and acoustic stimuli and shock duration, at the same time that it records the position of the experimental subject in each of the shuttle boxes, deciding about stimuli presentation accordingly. The data relating to each of the observed events are stored in results files that pick up the information acquired during the working session.

The program is capable of performing the procedure of yoked fully automatically. When working in this way, two cages are associated, one being the master and one slave. All stimuli received master's cage is also presented in the slave without the individual to alter its behavior (avoidance) what happens.

To operate, the program requires the previous existence of "Schedules". Schedules are a set-up of definitions related to the length of each event that occurs in the shuttle box and their respective consequences. The Schedules establish the conditions that must be met during a working session.

A "*classical*" terminology is used in this Manual when referring to stimulus and the responses emitted by the experimental subjects. It is obvious that this is not the only terminology possible nor is used by all the investigators in this field. When we refer to "*unconditioned stimulus*" and "*unconditioned response*", we refer to the set-up of environmental conditions that determine the appearance of an escape response. An escape response is understood as a response that forms part of the basic behavioral repertory of the experimental subject, independently from any previous experience. When talking about "*conditioned stimulus*" and "*conditioned response*" we refer, to the environmental conditions that produce the appearance of the avoidance responses when they are temporarily associated with the unconditioned stimulus and, on the other hand, to the avoidance response generated by this presentation.

2. INSTALLATION OVERVIEW

The installation procedure is completely described for reading during the installation procedure. Therefore, they are stand-alone and can be executed separately as per your needs: acquisition (USB Key must be connected), analysis, reporting (printer must be installed), etc.

First, 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 is done.

Additionally, you will find details about how to configure some requirements, which should be fulfilled to be able to install this system.

2.1. Requirements

SHUTAVOID needs the equipment bellow:

- A fully compatible computer with at least:
  - 2,2 GHz Pentium® processor (Celeron processor not supported).
  - 2 Gb of RAM.
  - HD 250 Gb (150 MB of free hard disk space).
  - Graphics: 1024x768 pixels and 32-bit true color.
  - 1 free USB port for the protection key.
- Connection interface:
  - 1 free USB port to connect the RS-232 converter for the boxes connection.
- Operating system supported:
  - Microsoft® Windows® 11 64bits.
  - Microsoft® Windows® 10 32bits / 64bits.
- Printer (advisable)

  At least one "virtual printer" must be correctly installed. Please refer to 2.6 for more details.

2.2. Installing the Software

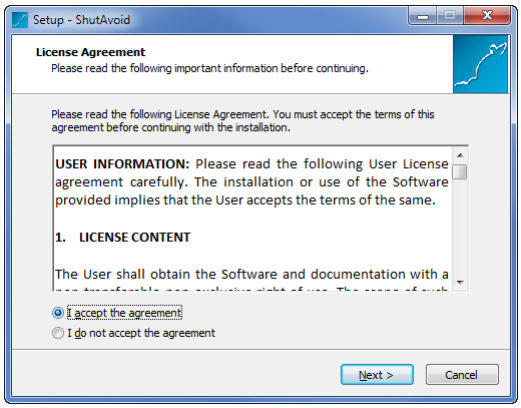
SHUTAVOID software is delivered within a single USB flash drive. The USB flash drive contains the software installation tool, this User's Manual in PDF format and other components required to work in specific conditions.

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.

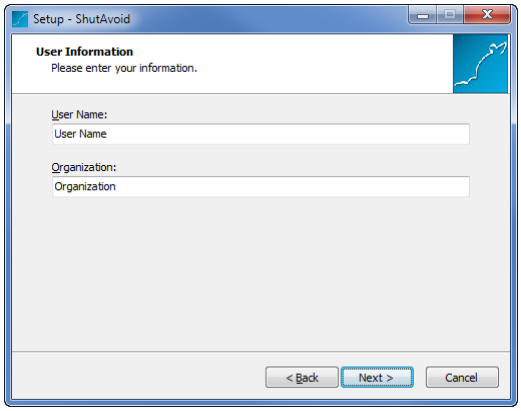
Once you get the administrative rights to install the software, please follow these steps:

- Plug the USB flash drive in a free USB port of your computer and wait until Windows® installs it as a new removable drive.
- Access the new removable drive detected and execute the PANLAB.EXE file. A window will be shown, as below:

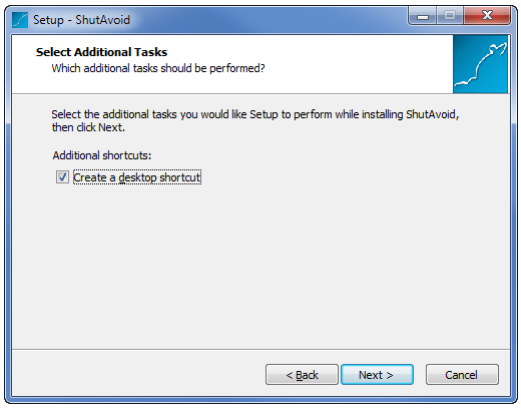
- Press the [Install SHUTAVOID v1.9.01] button to start the software installation.
- An installation wizard will appear. Select the "I accept the agreement" check button and press the [Next] button to start the software's installation.



- In the next windows introduce the name of the user and the company in the correct field. After this, press [Next] button to continue.



- During the installation process the software is installed in a new folder called [Panlab\SHUTAVOID v1.9] created under the Programs Files folder. If desired, the installation program allows you to choose another folder to locate the software. The location of the software is independent of the data folder, which is defined by the user using the corresponding options of the program.



- Press the buttons [Next] and [Install] following the Install Shield Wizard until reaching the [Finish] button.

- A new shortcut will appear on your desktop. Use it for executing the program later.

2.3. Installing software protection key

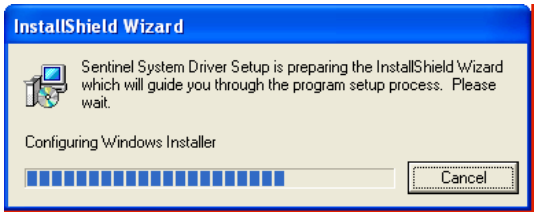
SHUTAVOID software is delivered with an USB protection key that avoids from fraudulent use of the application in a computer, which does not have it installed.

Before installing the drivers, all applications must be closed, and all USB SuperPro keys must be removed.

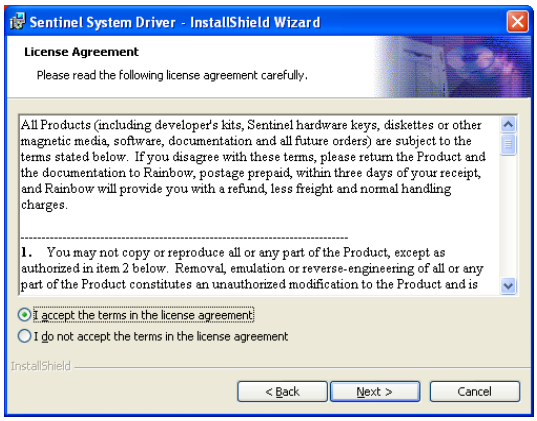
In order to do a correct USB key protection installation, please follow the steps below:

- Press the [Install Software Key Drivers] button to start the driver's installation.

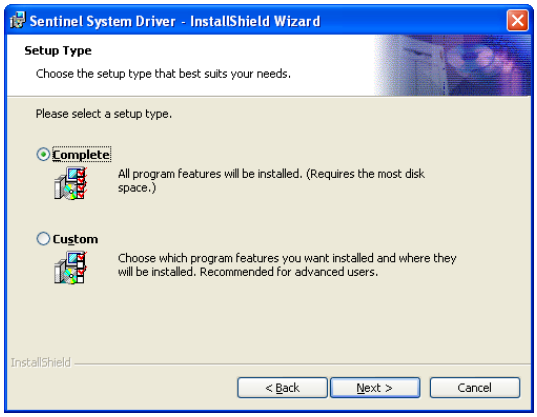
- Automatically a USB key wizard installation will be shown. When the [Welcome] screen appears, click [Next] to continue.



- Chose [I accept the terms in the license agreement] and click [Next] button to continue.

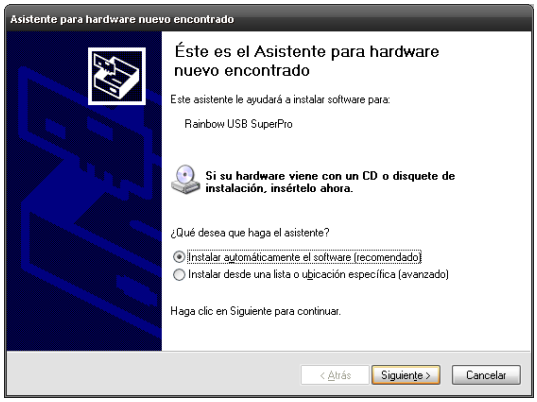


- On the incoming window, please select the [Complete] option of [Setup Type] window, click [Next] and [Install] buttons to continue.



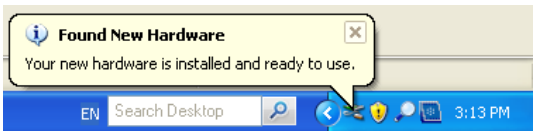
- After pressing [FINISH] button, you must reboot the system. Thus, your computer will recognize the USB security key.
- Did you reboot the computer? If you answer yes, please connect the USB key. In the lower right corner of the screen will appear the next message.

- The wizard for installing the drivers will run when your computer detected correctly the USB key. This process will need some minutes depending on your PC.
- Choose [Automatic Installation] and press the [NEXT] button.



- Wait while the wizard looks for the drivers until it asks you to press the [FINISH] button.

- Finally, a new message will appear in the lower right corner of your screen. The USB key was installed correctly...



- Important remark: This step has to be repeated for each USB port of your computer.

2.4. CONRS232USB-HS converter (high speed mode)

SHUTAVOID requires the use of the high-speed converter from RS232 port to USB port. A USB – Serial adapter will allow you to set 2 serial ports in your PC or laptop.

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

We recommend the use of a specific model of converter. We cannot guarantee a correct functioning of the system with any other USB-serial converter. The converter includes an extension cable just in case.

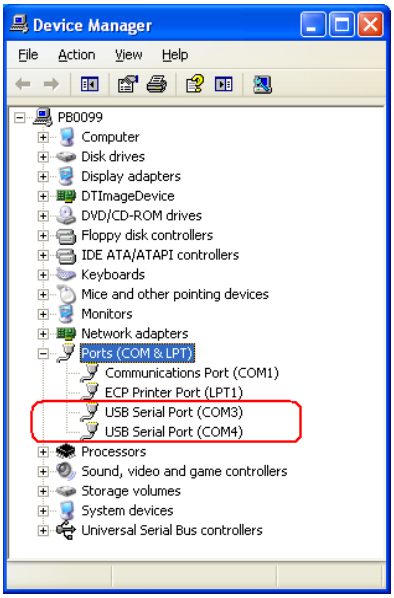
To Install the converter:

Connect the converter to the computer.

Windows 8, 10 and 11 will automatically install the drivers.

If working with a Windows 7 or previous, please refer to the notice provided in the box of the converter.

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.



2.5. MOXA converter (Legacy mode)

The MOXA converter was included in older SHUTAVOID software packages.

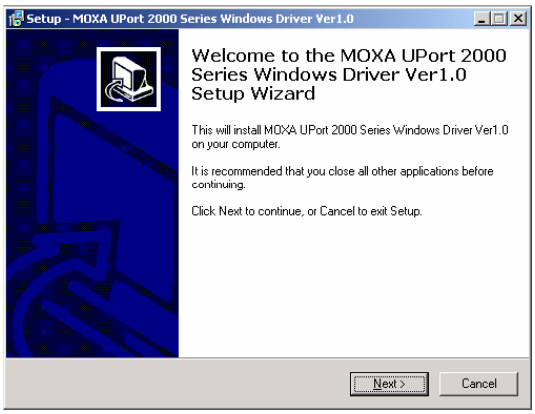
SHUTAVOID is still compatible with the use of the system in a Legacy mode (not high-speed).

Some computers could present an incompatibility with the MOXA USB-to-Serial converter device and the Shuttle boxes (because of electrical issues) provoking the application to crash during the execution of the trials. In that case, it is strongly recommended to connect the boxes directly to a standard RS232 port of the computer. If the computer has not any RS232 port, it is suggested to install an extension board, which can be easily found in most of the computer and electronic shops.

In case you need to re-install this device, please follow the below procedure:

- First, please check that your user has administrative rights on the PC or laptop in which the device is to be installed. Please contact your IT staff in order to clarify this issue before continue installing the device.
- Press the [Install Drivers USB-RS232 MOXA] button to start the driver's installation.

- In the welcome to install form, click **Next** to start installing the driver.



- A series of windows will show you to confirm the installation path and the start menu folder please click **Next** in these windows until the Completing wizard form appears. Then click **Finish**.

2.5.1. Connecting the Hardware

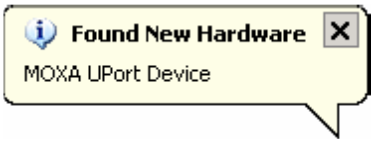
- Please install the driver before connecting the UPort 2000 to your PC's USB port. See the previous section for details.
- Use the proper serial cables to connect your serial devices to the UPort 2000's serial ports, which support the RS-232 interface. The UPort 2000's serial ports use DB9 male connectors with standard RS-232 pin assignment.

- The UPort 2000 has LEDs on the front panel. The "Active" LED indicates the power status, and the "TxD" and "RxD" LEDs indicate the transmission status of each serial port. In addition, when you click "Locate" for a particular UPort, that UPort's "Active" LED will blink to indicate its location.

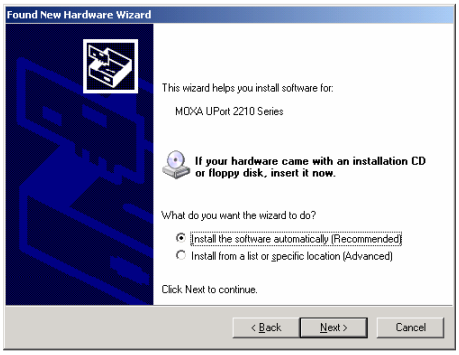
LED Name	LED Color	LED Function
Active	Green	Power is on

	Off	Power is off, or power error condition exists
TxD, RxD	Orange	Port is receiving data from attached device
	Green	Port is transmitting data to attached device
	Off	No data is being transmitted or received

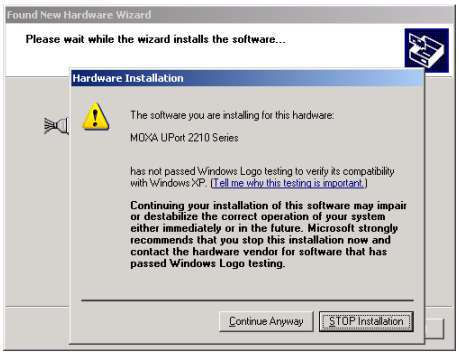
- After connecting the USB cable from UPort to host the PC, Windows will automatically detect the new UPort, and the **Found New Hardware** balloon will open in the bottom right corner of the Windows desktop:



- The wizard for installing the drivers for the [Found New Hardware] will run. Select the option [No, not this time] and press the [Next] button to continue. In the next window choose [Install the software automatically] and press the [Next] button again to continue.

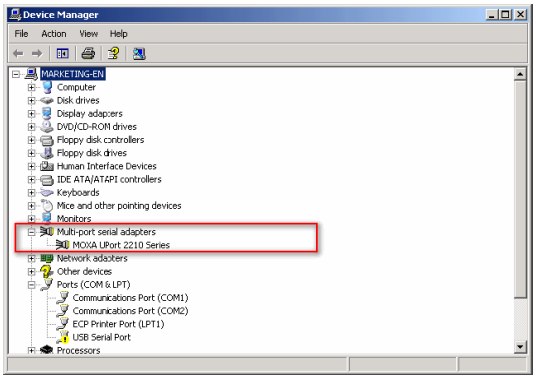


- Wait while the installation wizard searches for the correct drivers. The next window that opens cautions you that although this software has not passed Windows logo testing, this driver has already been tested and shown that it can support the Windows operating system. Click **Continue Anyway** to proceed.



- The next window shows the model name of the board and indicates that Windows has completed the driver installation. Click **Finish** to proceed with the rest of the installation procedure.

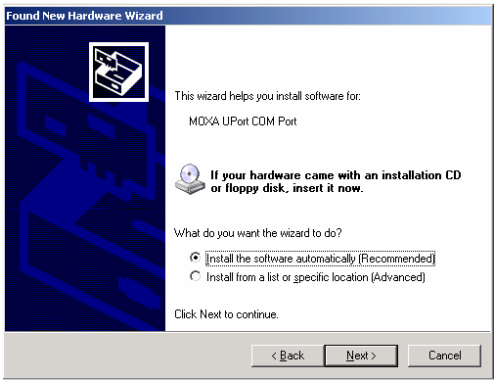
- Open the Windows Device Manager to check that the installation was successful. The UPort USB-to-serial converter should appear under **multi-port serial adapters**.



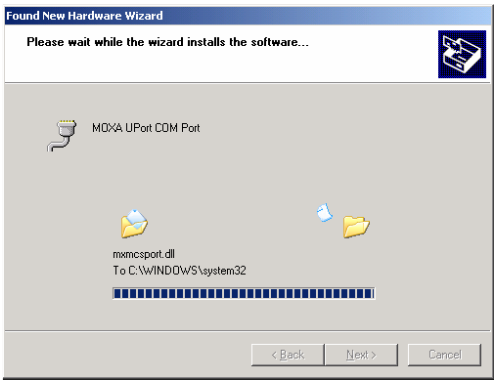
- Please, wait while the wizard locates the drivers installed previously. This process may require some minutes depending on your PC.

2.5.2.Installing the Driver for the Ports

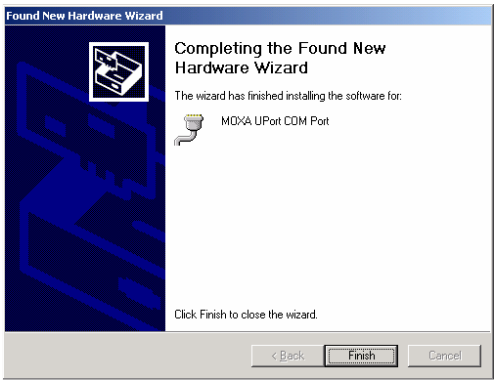
- The Found New Hardware Wizard window will open to help you install the driver. This window will offer to connect to the Windows update site to search for a driver. Select No, not at this time and then click Next to continue. In the next window choose [Install the software automatically] and press the [Next] button again to continue.



- Wait while the driver is installed.



- After all files have been copied to the system, the Completing the Found New Hardware Wizard window will open to indicate that it has finished installing driver. Click Finish to proceed with the rest of the installation.



- Repeat the last 3 steps for each of the remaining ports (1 port for the UPort 2210).

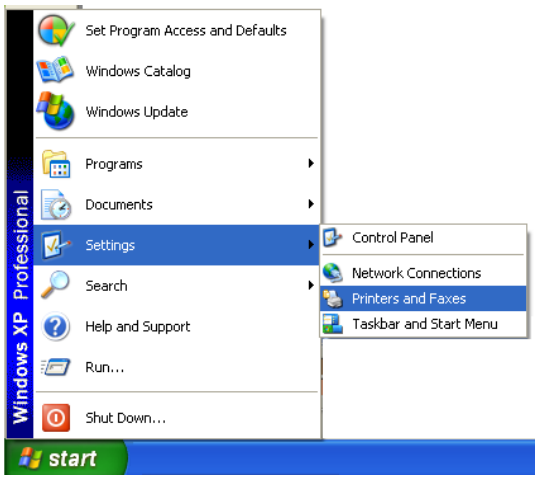
Important remark: Until now, only one serial port has been correctly installed. The process must be repeated for the second port. Please, wait while your PC or laptop finds another COM port. Once again, the next message will appear in the lower corner of the screen:

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

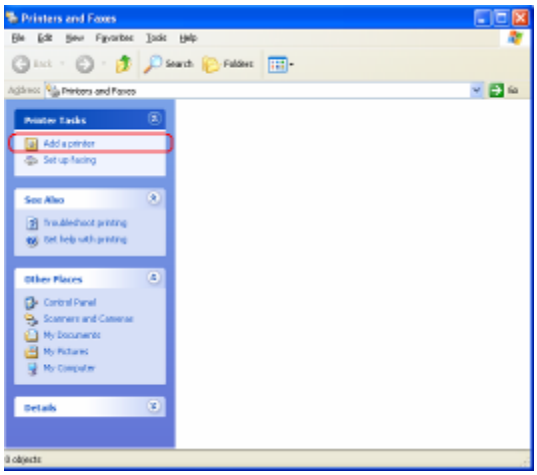
2.6. Installing a printer by default

If there is not a printer installed in your PC, one virtual printer must be installed by default. If your PC is running Windows 10 or superior, there are two virtual printers installed by default (Microsoft XPS Document Writer and Microsoft Print to PDF) and no additional action is required; but for earlier Windows versions, the next steps must be followed to fulfil the system requirements:

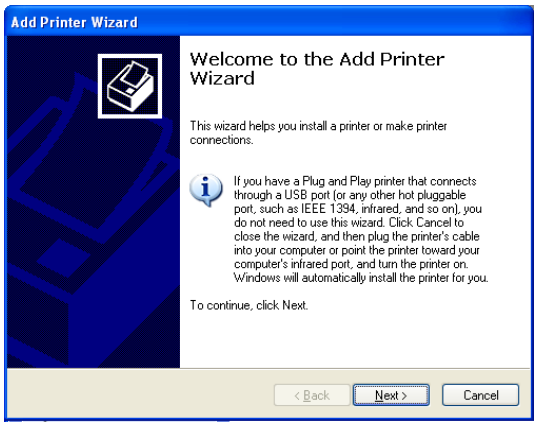
- Go to [Printers and Faxes] option of your system. The access is possible by clicking on [START – Settings].



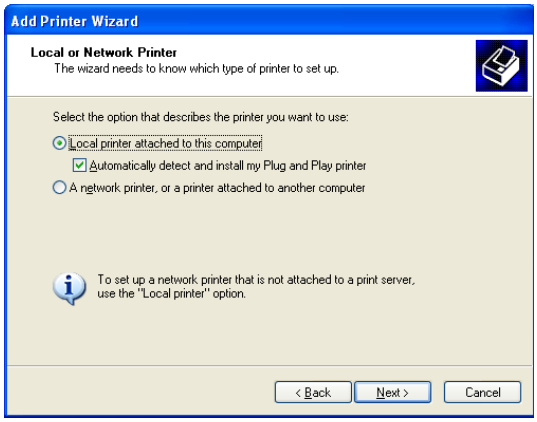
- In the [Printers and Faxes] window press on [Add printer] button.



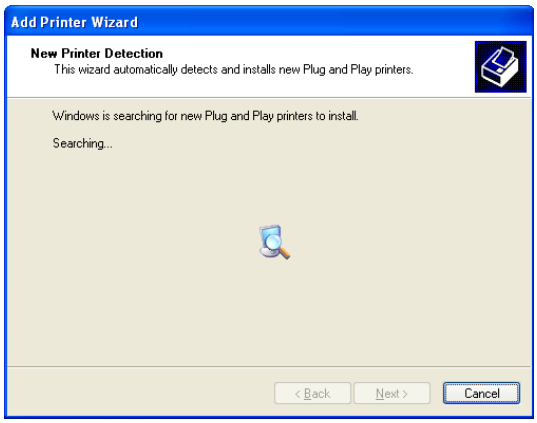
- The Welcome to the add printer wizard appears, click [Next] to continue.



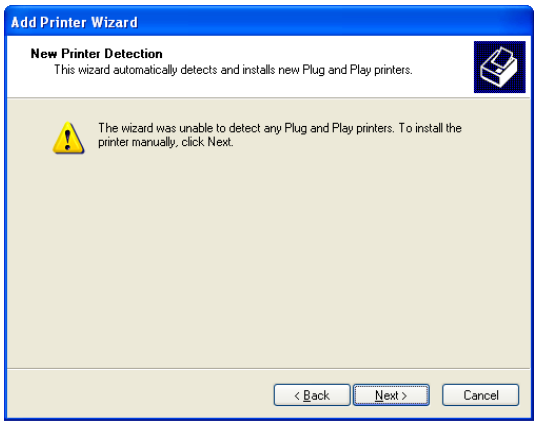
- As this procedure is for installing a virtual printer, the options must be selected as is shown in the next window. Press [Next] button to continue.



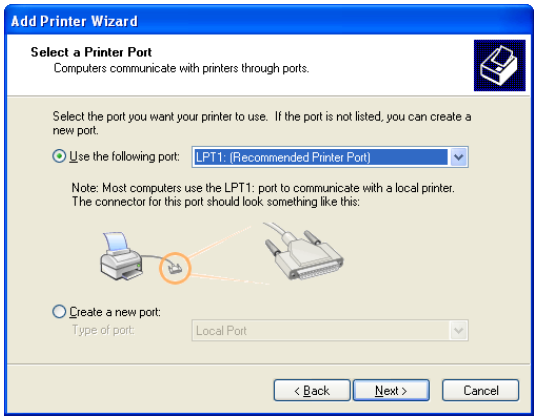
- The wizard will search for the drivers for the virtual printer. This process will need some minutes depending on your PC.



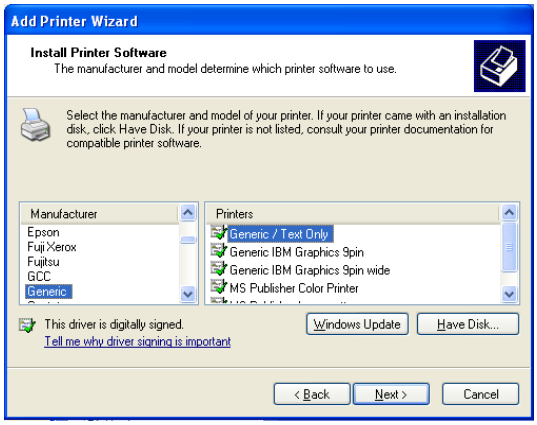
- The wizard notifies that there is not a real printer present. Press [Next] button to continue.



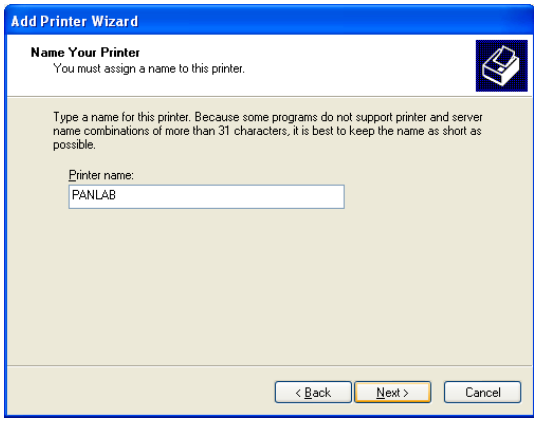
- Obviating the previous message, the options for the next window must be selected as is shown in the next picture before pressing the [Next] button.



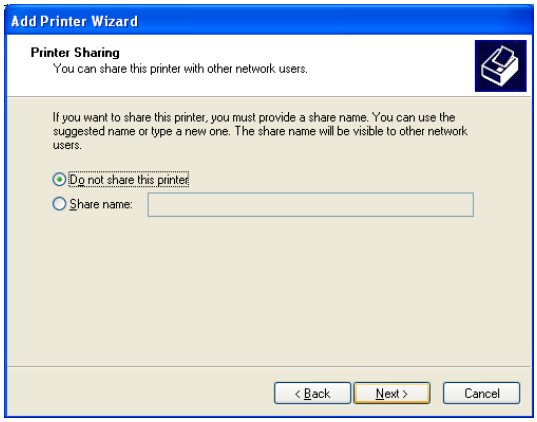
- The virtual printer will be a [Generic / Text Only] thus these options must be selected in the next window before press the [Next] button.



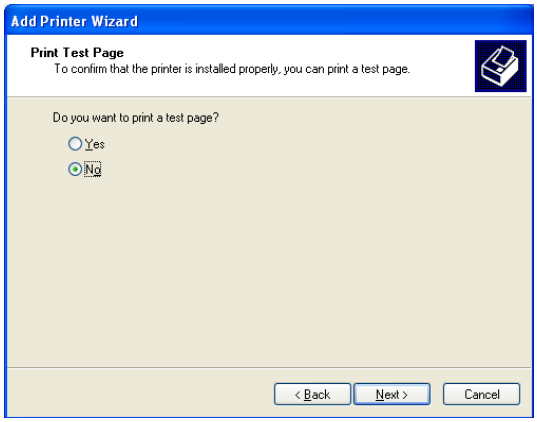
- This virtual printer can be called [PANLAB] thus it can be easy identified when a real printer will be connected to the system. The option [No] must be selected before press [Next] button.



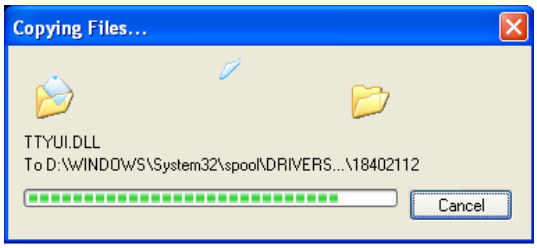
- Of course, is not necessary to share PANLAB printer thus select the [Do not share this printer] and press [Next] button to continue.



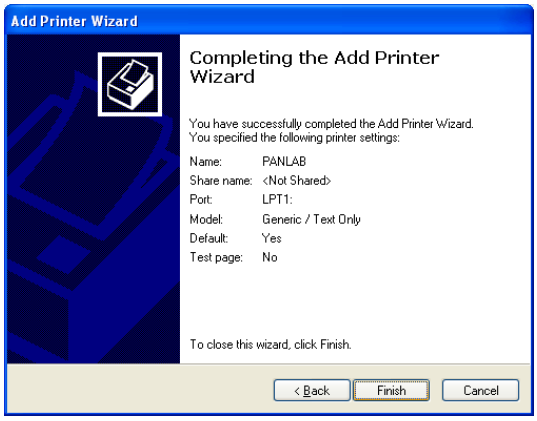
- Neither is not necessary print a test page thus selects [No] option for answering to the wizard and press [Next] button to continue.



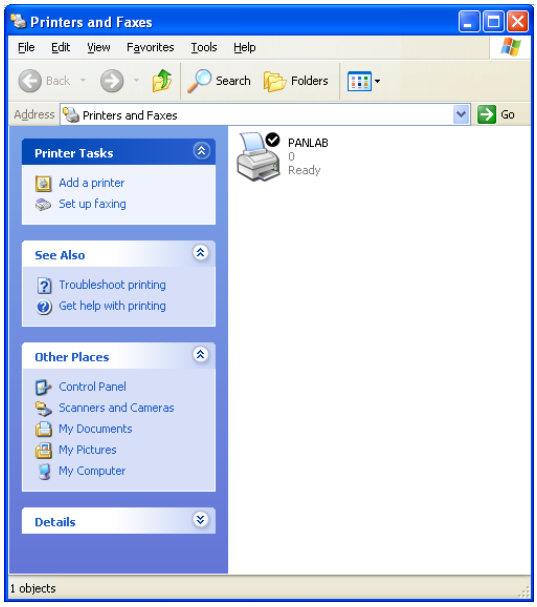
- The wizard will copy the drivers for the virtual printer. This process will need some minutes depending on your PC.



- When the virtual printer is successfully installed, the wizard shows you a window as the next picture. Press [Finish] button.



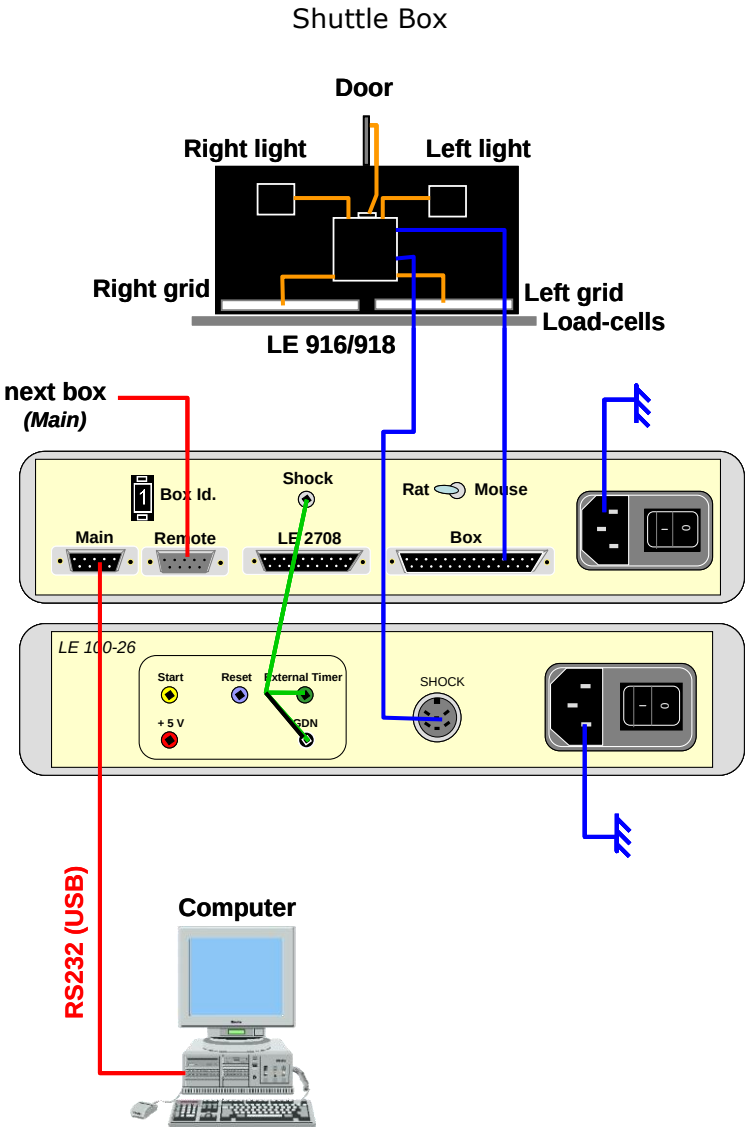
- A new icon will appear in [Printers and Faxes] window called [PANLAB]. Close the window and launch the software.

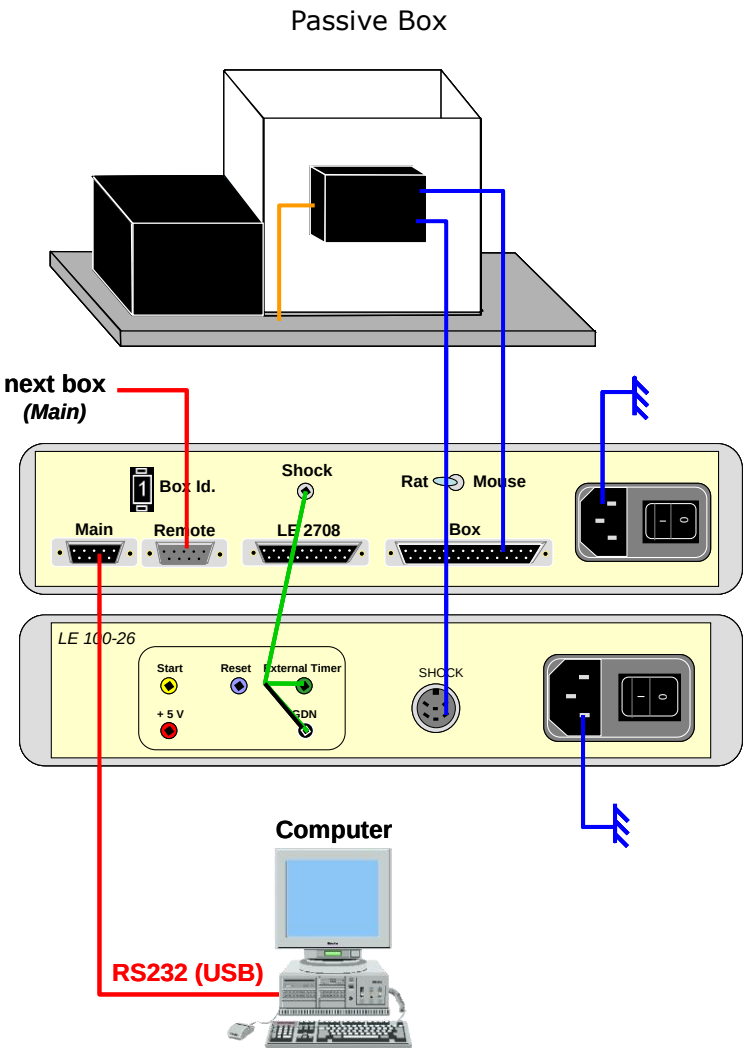


2.7. Hardware Installation Guide

2.7.1. Chamber Connections

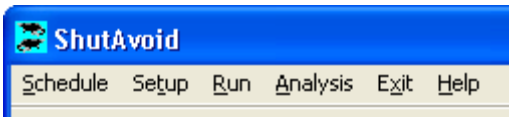
PC using SHUTAVOID program can control the experimental chamber. The following connections must be made between each chamber and its associated control unit and shocker LE 100/26. The different experimental chambers (up to four chambers) are connected together in series with the first one directly linked to the computer thought the RS232 Serial Port (or a USB port by means of the optional Serial-USB adapter).




Passive Box
next box
(Main)
Box Id.
Shock
Rat
Mouse
Main
Remote
LE 2708
Box
LE 100-26
Start
Reset
External Timer
+ 5 V
GDN
SHOCK
Computer
RS232 (USB)

3. The main menu

Once the program starts, the experimenter is presented with a "Main Window", in which the "main menu" is shown. The options of this menu are presented in corresponding line of the screen.



The main menu (see figure above) allows for the selection of the following options:

- Schedule

This option allows opening a window in which it is possible to define the conditions in which the experimental subject stimuli will be affected as well as the data referring to the shock's characteristics. The sets up of experimental conditions are filed in a specific archive under a SCHEDULE NAME from which they can be recuperated for use in an experiment.

- Setup

Using this option the user can either define the paths to be used for filing or check the correct wiring of the experimental setup.

- Run

This option opens a window in which full control to carry out of an experiment is provided.

- Analysis

This option allows configuring the data analysis and gives access to experimental data in numeric format. Data exporting and printing procedures are also included here.

- Exit

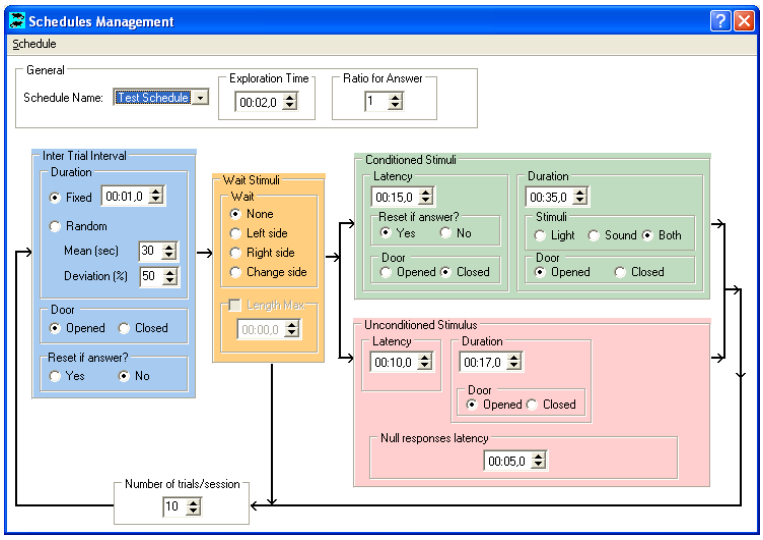
This option finalizes the program execution.

- Help

This option provides fast and easy access to the user manual in PDF format. That is a copy of this paper version.

4. The Schedule option

Using this option a window like those shown below is open. In this window, all the parameters forming a schedule definition are presented.



The different parameters forming the schedule are fully described in the next sections of this manual.

Clicking on the upper left corner the pop-down menu of [Schedule] will be shown. This menu controls the basic operation to be carried out when defining a Schedule.

- New

Allow to create a new schedule, whose name is requested in a dialog window. The default values are loaded and appear filling the fields of the new schedule.

- Rename

After the new name is entered in an appropriate dialog window, the schedule currently shown is renamed (with a non-existent name). This makes the old schedule no longer available using the old name.

- Save

Allow to save the current schedule using the values present at the window.

- Save as...

Allow to make a copy of the current schedule saving it with a new name. The old schedule is still available under the former name.

- Reset

Clears the parameters of the current schedule and loads the default parameters.

- Delete

Allow to delete the current schedule. There is no way to recover an already erased schedule.

- Printer Font

Allow to select the font to be used by the printer when printing schedules.

- Print

Activates a small menu allowing selecting either print, the current schedule or printing all of them.

- Exit

Closes the Schedule's window and turn back the control to the main screen.

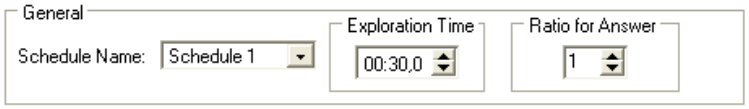
The schedules are stored in a specific file called "*Schedule3.sch*". This file must be always located in the same directory as SHUTAVOID and if removed, all this information will be lost.

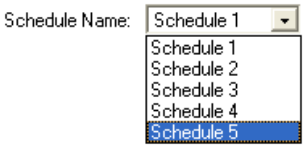
4.1. Schedule parameters

The following sections describe the parameters forming a schedule definition.

4.1.1. General Information

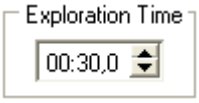
Under this title some general information about the schedule are included:





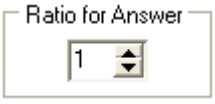
- Name of Schedule

Is a text that identifies a Schedule; this name must be new for each new Schedule; no two Schedules should have the same name. Opening the list by pressing the down arrow at the right (see figure at the left) allows for the selection of a schedule from the schedules currently available. Use [New] option for creating a new schedule's name.



- Exploration Time

During this time, the experimental subject is left in the shuttle box to explore freely. The number of changes of position of the experimental subject within the box are recorded and reported, but they do not affect the later development of the experimental Schedule.

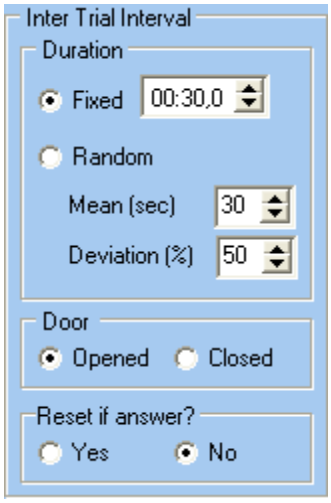


- Ratio for answers

This is the number of times that the subject must change position in the box to reach a valid response. The maximum number of acceptable changes is 999. This parameter permits the establishment of two concepts, which are evaluated separately: the change of position in the box and the valid response; this last must be understood to be a set of changes (that can have a unitary value).

4.1.2.The Inter Trial Interval (ITI)

This is the period between the beginning of a trial and the presentation of the acoustic and/or visual stimuli. The length of this period (hh:mm:ss) can remain constant throughout the trials or vary from one trial to another in a random way, accordingly to those state by the parameters described below.



- Duration

Allow to establish the length of ITI. If the "Fixed" option is selected, the ITI duration will be always the same (those corresponding to the value currently in the hh:mm:ss window). If the "Random" option is selected, the duration of each ITI will be different, varying according to the settings of the "Mean" and "%" windows.

When setting the duration as "Random", it is necessary to define the Mean duration (sec) and its maximum deviation (%). In this mode, the ITI of all the trials will have an average equal to the defined Mean and with extreme values above or below the lower and upper limits respectively. The probability of the occurrence of any of the values in the valid interval is the same, so that a uniform and not normalized distribution (not Gaussian) is obtained.

- Door status

Allow to establish the status of the guillotine door (open/closed) at the beginning of the ITI.

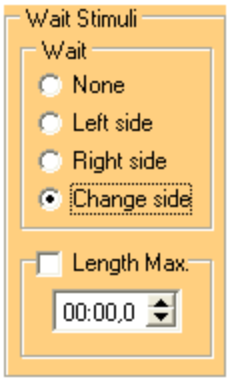
- Reset if Answer?

Select for the effect of a correct answer detected during this period. If "Yes" is active, the answer will produce the reset of the current trial and the beginning of a new one. If "No" is selected, the answers produced during this period will be recorded but it will have no consequences on the development of the current trial.

A response produced in this period is called "*anticipation response*" (also "*superstitious response*").

4.1.3.Wait Stimuli

This section establishes the conditions for transition to the stimulus, once finished the ITI.



- Wait

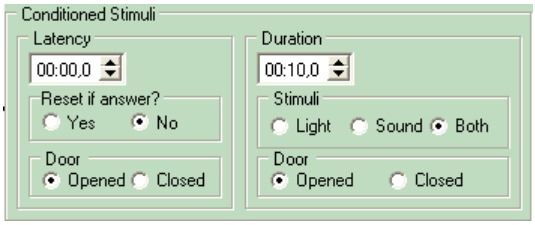
  Let us fix that will happen in the cage to get through to stimuli. In a classic active avoidance protocol, status is "*None*". In other cases, it is expected that the subject is in either side or that side effect a change, the maximum waiting time specified in [Length Max] configuration.

- Length Max

  Set the maximum time the system waits for the selected condition to be met in the previous point (Wait). If the option is selected, then wait a maximum time selected, or if selected, will wait all the time that the selected condition is met.

4.1.4.Conditioned stimuli

In this section, the parameters affecting the conditioned stimulus are defined:



- Latency

  Allow to define the time that passes between the end of the intertrial period and the beginning of the conditioned stimulus.

- Latency - Reset if Answer?

  Select for the effect of a correct answer detected during this period. If "Yes" is active, the answer will produce the reset of the current trial and the beginning of a new one. If "No" is selected, the answers produced during this period will be

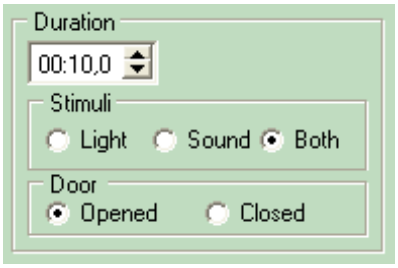
recorded but it will have no consequences on the development of the current trial.

- Latency - Door Status

  Allow to establish the status of the guillotine door (open/closed) during the Latency time before the conditioned stimulus.

- Duration

  Is the time (hh:mm:ss) during which the conditioned stimulus is presented.



- Duration - Stimuli

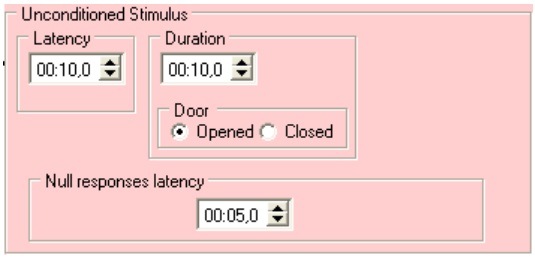
  Allow to define the element(s) forming part of the conditioned stimuli (light, sound or both), according to the active button.

- Duration - Door Status

  Allow to establish the status of the guillotine door (open/closed) at the beginning of the presentation of the conditioned stimuli.

4.1.5. Unconditioned stimulus

Set for the values of the different parameters defining the unconditioned stimuli (electric shock). See next page for the appearance of this part of the definition.



- Latency

  Allow to define the time that passes between the end of the intertrial period and the beginning of the presentation of the electric shock.

- USD - Unconditioned stimulus duration.

  The shock remains active during that period.

- NRL - Null responses latency.

  This is the maximum period in which the program waits for the appearance of a valid escape response. Its maximum length is the shock duration (in this case, ALL the responses emitted during its presentation are considered valid escape responses); the minimum is zero, in which case there are not any escape responses that can be considered valid. The escape responses that are produced after the escape response latency has elapsed are considered "*null*" responses.

- Door Status

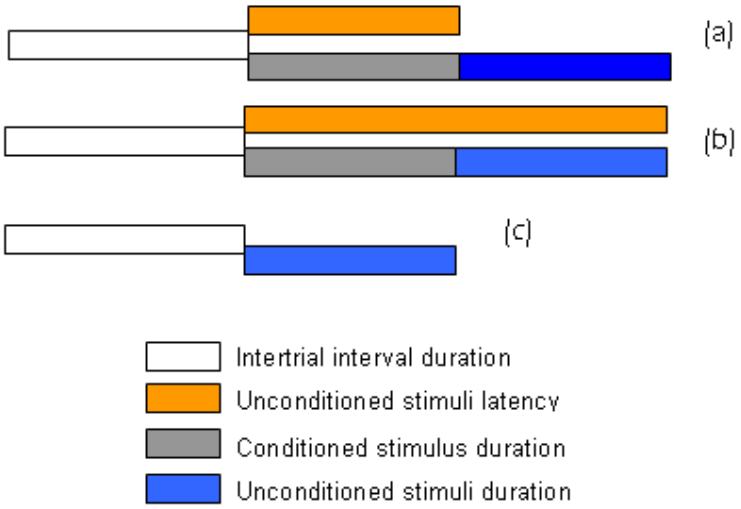
  Allow to establish the status of the guillotine door (open/closed) at the beginning of the presentation of the conditioned stimuli.

4.1.6.Trial repetitions

This is the number of times that the ITI-USL-USD sequence must be carried out during an experimental session.

4.2. Timing a schedule

The following is a scheme of the temporal parameters forming the definition of an experimental schedule. It is important to note certain inherent limitations to such temporal relations.



In the first case (a), the length of the unconditioned stimuli latency and the duration of the unconditioned stimulus are the same. This makes the unconditioned stimuli presentation occur once the conditioned stimulus is over.

In the second (b), the duration of the conditioned stimulus equals the unconditioned stimuli latency plus the unconditioned stimulus duration, making the shock to be superposed with the light/sound.

In the third (c), the duration of the conditioned stimulus and the latency of the unconditioned stimuli are zero. As a result, an unannounced shock is presented at ITI intervals.

If the duration of the conditioned stimulus is longer than the latency of the unconditioned stimulus plus the duration of the conditioned stimulus, an uncommon situation arises, as the conditioned stimulus will be activated even after the stopping of the shock.

In general, this procedure to define a schedule allows for a very high degree of flexibility. Some "uncommon" situations (such as those described in the precedent paragraph) are advised. Nevertheless, the main responsibility of the coherence of the schedule's definition is entirely in the hands of the user.

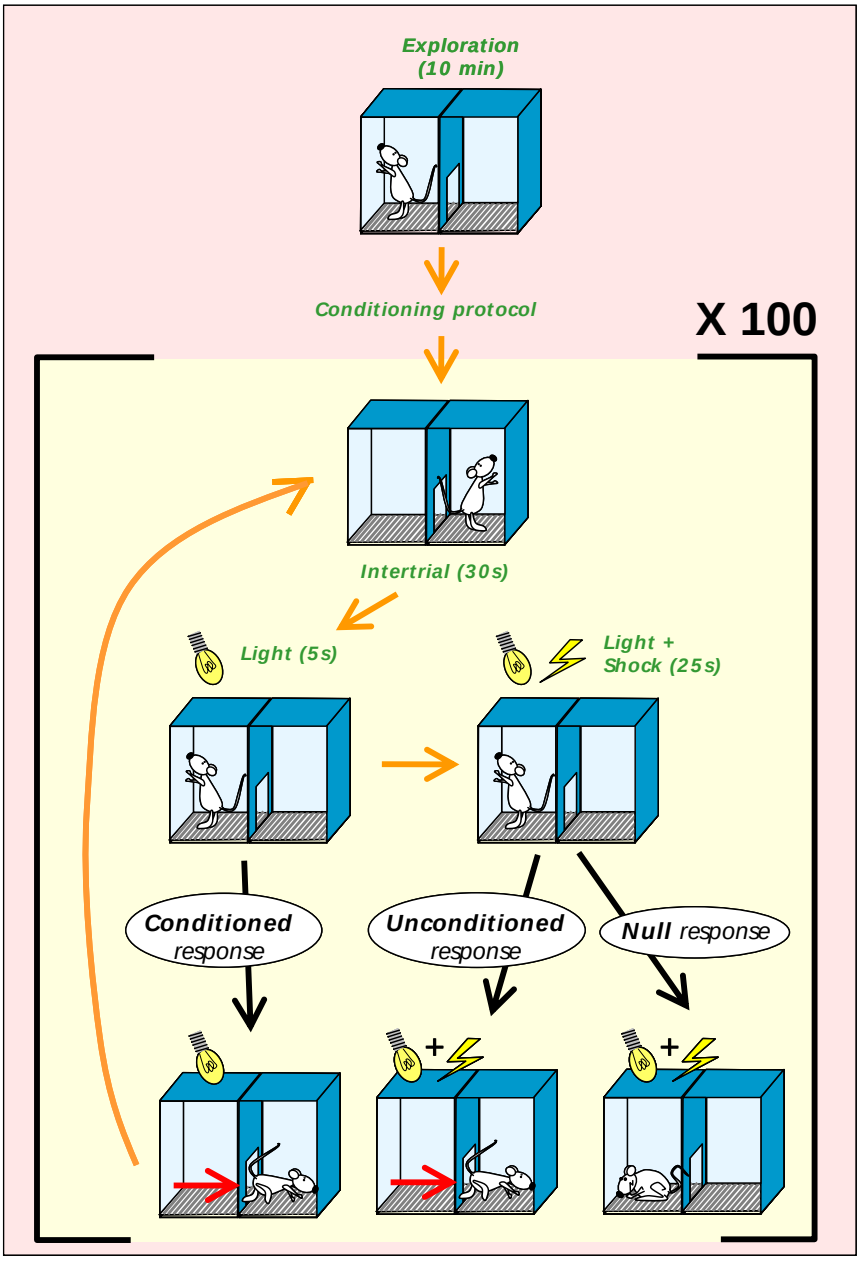
4.3. Example of schedule for active and passive avoidance paradigms

The SHUTAVOID Software has been built to allow configuration of both active and passive avoidance experiments.

Both active and passive avoidance are fear-motivated tests of learning and memory that are classically used in experimental subjects such as rat and mice.

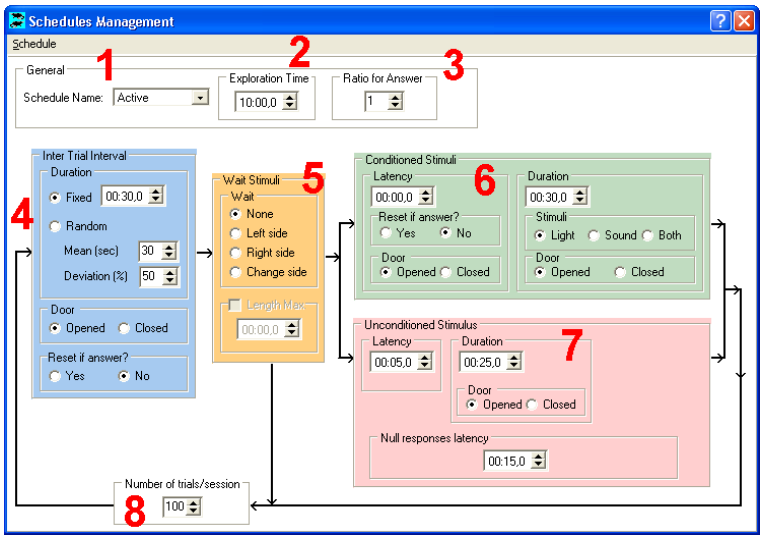
A wide variety of protocols has been reported in the literature depending on the experimental subject specie, strain, laboratory, aim of the study etc. Here, are given two examples of protocols and the corresponding schedule configuration with SHUTAVOID.

4.3.1. Active avoidance



Example for mice: Briefly, the test is performed in a shuttle avoidance apparatus. The experimental subjects are first allowed to explore the box for 10 minutes. They then receive 100 trials per day for 5 consecutive days, a total of 500 trials with an inter-trial interval of 30 sec. In each trial, the experimental subject will be presented to a conditioned stimulus and an unconditional stimulus. The conditioned stimulus (CS) consisted of a signal light for 30 seconds (may also be a sound or both), preceding the unconditioned stimulus (US; continuous electric stimulation through the grid floor; duration: 25 seconds) for 5 seconds. For the US, mild electric stimulation has to be carefully chosen to minimize the occurrence of freezing (for mice, around 0.2 mA). Both CS and US are terminated when the experimental subject ran to the other side of the apparatus. Are evaluated the number of Conditioned responses, Unconditioned response and Null response (see next figure).

In SHUTAVOID, [Schedule] option has to be selected in the Menu and configured as follow:



1. General

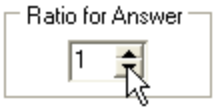
   Name the protocol (example: "Active").

2. Exploration time

   In the example, the value for [Exploration time] is 10 min.

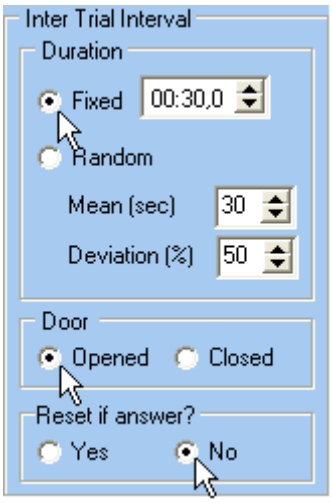
3. Ratio for Answer

The change of compartment of the subject has to be considered as response. The [Ratio for Answer] is 1.



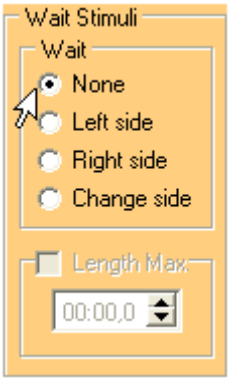
4. Inter Trial Interval

- The Inter Trial Interval Duration is of 30s.
- During the exploration period, the door should be [Open] selected.
- Reset if answer has to remain unselected [No]

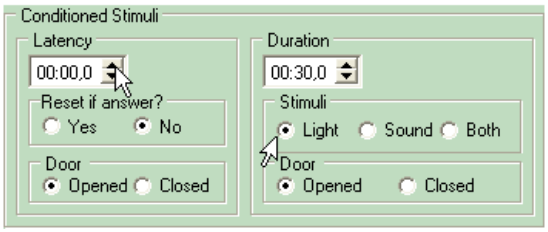


5. Wait Stimuli

The Wait Stimulus option is not used in the active avoidance paradigm. [None] has to be selected.

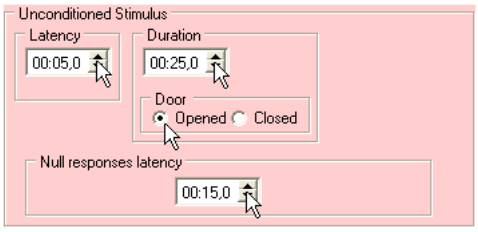


6. Conditioned Stimuli
   - The Latency is set at 0 sec.
   - Select [No] option for "Reset if answer?"



   - During the latency period, the door should be opened.
   - The Duration of the Conditioned stimuli (Light) is 30 sec.
   - The door has to remain Opened during the Conditioned stimuli.

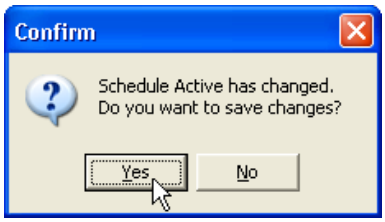
7. Unconditioned Stimulus
   - As unconditioned stimulus, the experimental subject receives an electrical shock (*Latency* = 5 seconds, *Duration* = 25 seconds).
   - The *Door* has to remain *Opened*



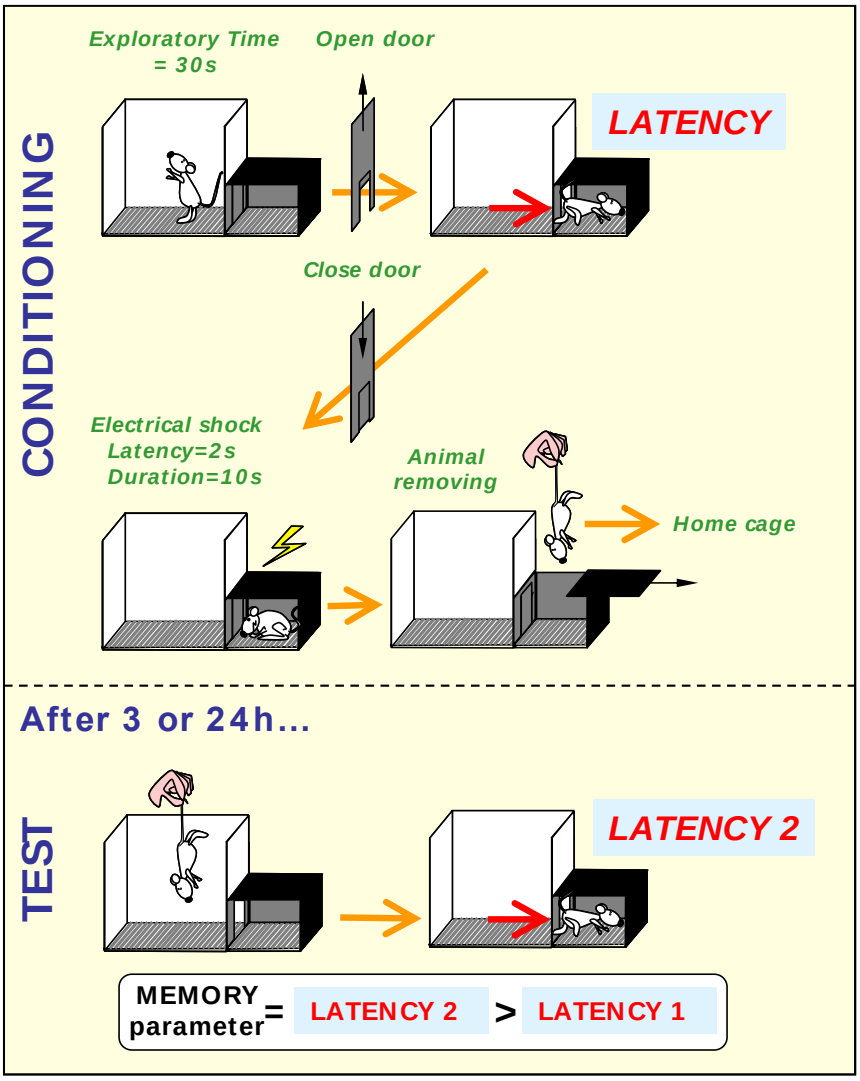
   - In our example, if the experimental subject does not change of compartment within 10 seconds after receiving shock, (e.g. 15 s after light ON), the response is "*Null*". The *Null response latency* is then of 15s.

8. Number of trials/session
   - 100 trials are performed for each experimental subject (=100).

   - Save the protocol by pressing [Yes] button.



4.3.2. Passive avoidance

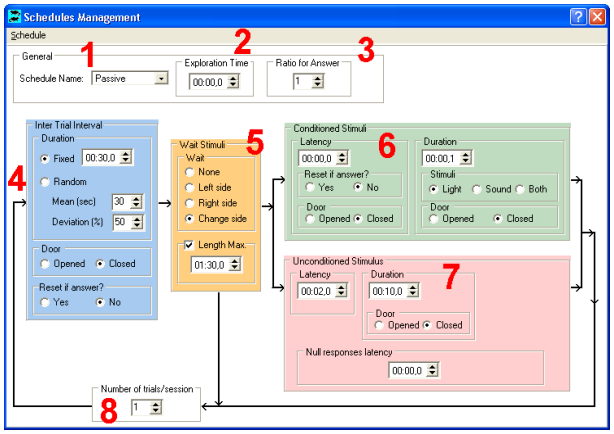


Example for mice (see also next figure): Originally, the apparatus consists of a large, white-painted illuminated compartment and a small, black-painted dark compartment separated by a guillotine gate, but the experiments can also be performed by using a shuttle box. The test consists in two phases: a Conditioning phase followed by a Test phase. During the Conditioning phase (one trial), the experimental subject is placed in the illuminated compartment and allowed to explore it for 30 seconds, leaving the guillotine gate closed (Exploration period). At the end of the exploration time, the guillotine gate is opened and the time elapsed before entering into the black chamber is recorded. If the experimental subject did not enter in the black compartment before a defined time (Cut-off latency = 90 seconds), the experiment is aborted and the experimental subject eliminated from the experiment. As soon as the experimental subject enters into the dark compartment, the guillotine is lowered. After a latency (generally short, here = 2 seconds), a strong electrical

shock (0.8 mA) is given through the grid floor for 10 seconds. Immediately after receiving the shock, the experimental subject is removed from the black chamber, returned to its home cage, and the chamber is thoroughly cleaned.

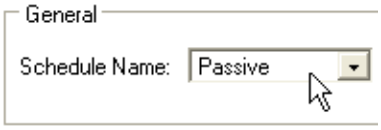
The test phase is performed 3h (short-term memory) or 24h (long-term memory) after conditioning. The experimental subject is placed again in the illuminated/white compartment for some minutes and the latency between door opening and entry into the dark compartment is measured. Better memory performance is indicated by longer latency to enter in the black compartment in the Test phase than in the Conditioning phase.

In SHUTAVOID, [Schedule] option has to be selected in the Menu and configured as follow:



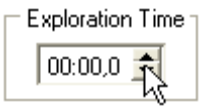
1. General

   Name the protocol (example: "passive").



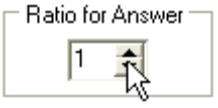
2. Exploration time

   In our example, the Exploration time (=30s) will be entered in **section 4. (Inter Trial-Interval)**



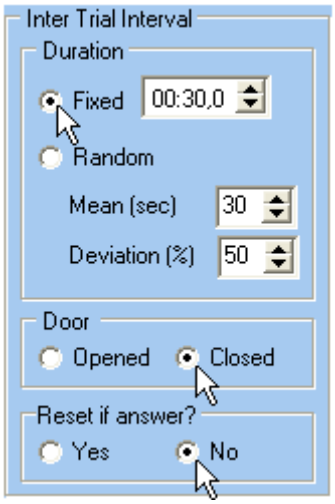
3. Ratio for Answer

   When the experimental subject changes of compartment has to be considered as response. The *Ratio for Answer* is then =1.



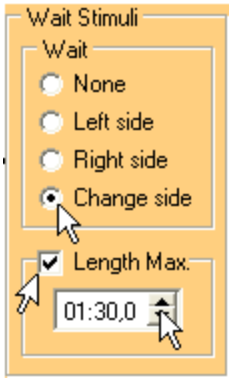
4. Inter Trial Interval

   - The Inter Trial Interval ***Duration*** can be used as exploratory time. The time of exploration is fixed (=30s).
   - During the exploration period, the door should be ***closed***.
   - The selection of "No" or "Yes" in the ***Reset if answer*** is not important. As the door is closed, the experimental subject can't change of compartment. So, no answer can occur during the Inter Trial Interval.



5. Wait Stimuli

   - When the exploration period is finished, a stopwatch will count the latency until an answer occurs. In our experiment, an answer corresponds to the experimental subject's change of compartment (***Change side***).
   - When the program enters in the Wait stimuli, the door will open automatically.



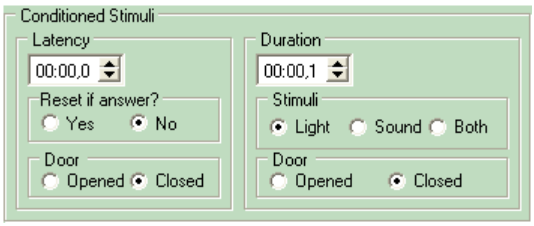
   - A ***Length Max*** is chosen as cut-off time (90s). If experimental subject did not change of compartment before this time, the experiment will automatically stop.

6. Conditioned Stimuli

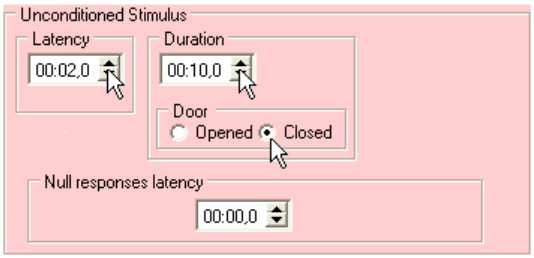
   - The ***Duration*** of 100msec was chosen in the Conditioned stimuli to allow the closing of the door once the

experimental subject had entered in the black compartment (***Closed***).

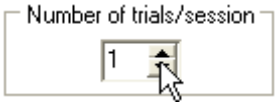
- There are no conditioned ***Stimuli*** in our experiment. As the passive box did not have any light or sound, no stimuli will be received.



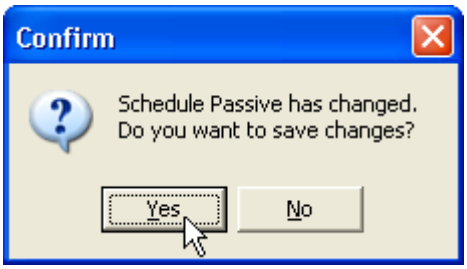
7. Unconditioned Stimulus
   - As unconditioned stimulus, the experimental subject will receive an electrical shock (*Latency* =2s, *Duration* =10s).
   - The *Door* has to remain closed.
   - The *Null response latency* option is not used in the passive avoidance protocol.

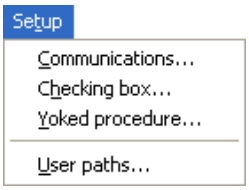


8. Number of trials/session
   - Only one trial is performed for each experimental subject (=1).



   - Save the protocol by pressing ***OK***.





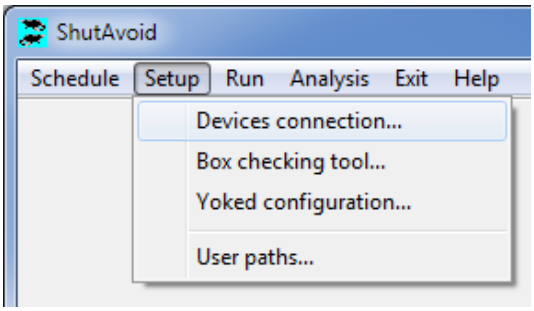
5. Setting up the system

By clicking [Setup] option, it is possible to configure the communications with the boxes, verify the correct functionality of the full system, to fix the working mode of the boxes and to establish the paths for the main files to be used when carrying out experiments.

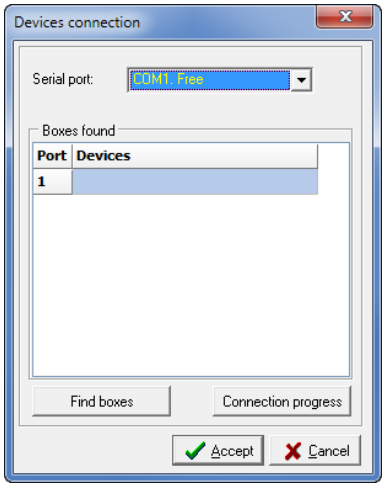
5.1. Setting up communication ports

The communication between computer and box is made through a serial port. This connection must be configured before starting the data acquisition the first time. To do so please see picture below:

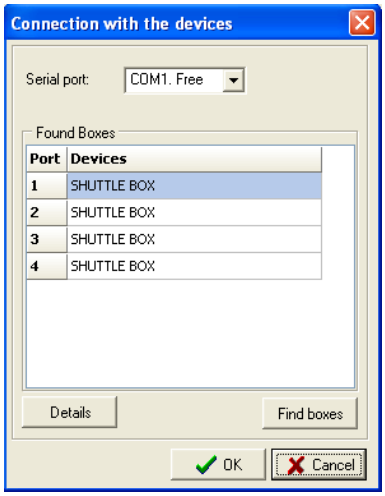
- Select [Setup – Device connection…] menu option



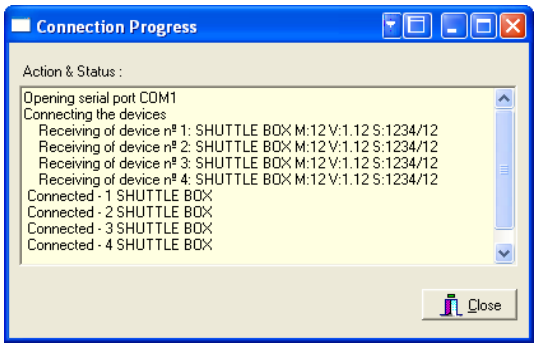
- In the "Boxes definition" window, press the "USE REAL CONNECTED BOXES" button.
- In the incoming window, select a free COM port available.



- After selecting the COM port, you can make a test to be sure that the connection between the computer and the box is ok by pressing [Find boxes] button. You will see the picture below:



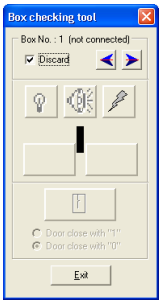
- If you press [Details] button, you will see the connection details (see picture below):



5.2. The checking box

The checking box is a tool designed for the verification of the correct functioning of the system (see below).

In the upper part, the box number appears. Using the right/left arrows, it is possible to select all the cages from the 1 up to the 8. If the "Not present" button appears checked, this means that the software is unable to find the respective box (see left below). If the box is present, the rest of the buttons appear as active and the box can be verified.



It is not possible to begin the verification without an object being present in the grids of the cage. Choose some adequate object (with a weight comparable to those of your experimental subjects) and place it into one of the sides of the box.

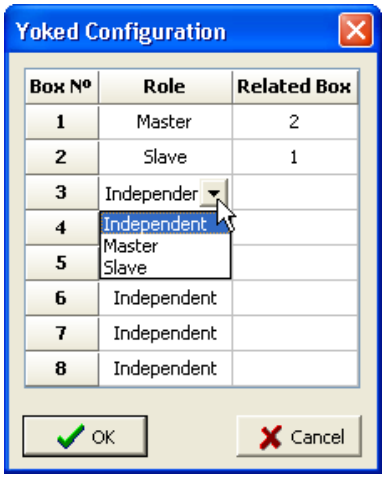
Doing this, the red led of the corresponding side in the Shuttle Box should shine and the checking panel of the software must also show the active side (by a black rat). When displacing the "rat" from one side to the other, this must be reflected consistently both in the software and in the shuttle.

By pressing the corresponding buttons, the light, sound and shock must be activated and remain active till inactivated by pressing the buttons again. The door button must be pressing (or pressing the door itself); cause the door to open or close.

If some of these functions are not carried out correctly, a malfunction should be suspected. The wiring should be checked; if the problem still subsists, the technical service should be called.

5.3. Yoked procedure

SHUTAVOID is capable of executing a protocol in yoked so without making any changes in wiring cages, only has to set the role of the cages.



Cages must be operational and that the dialogue of "Yoked configuration" cages are only detected and the user has configured to use them (the Discard unmarked Checking box dialogue).

A cage can have the following roles:

- Independent

  In this way the cage running a protocol without affecting another cage and without it being affected by another.

- Master

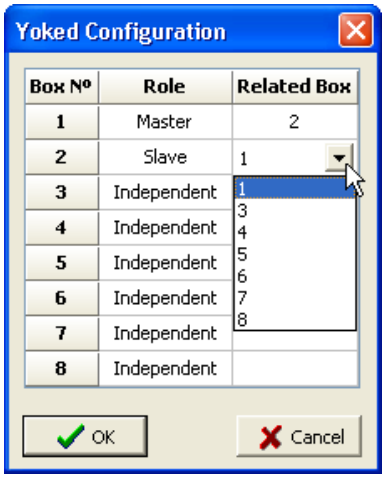
  The cage is running the protocol to be assigned without being affected by another cage, but if your cage affecting related (a slave). All stimuli received master's cage, also presented in the cage slave.

- Slave

  The cage is a cage-related Master. All stimuli presented in the cage master, also presented in the slave, but that the behavior of the slave to anything affecting the evolution of the protocol.

To determine the role of a cage as master or slave, there must be cages with the role of independent, so that the first independent cage becomes the related cage.

To change the cage connected to a master or slave, press under "Related Box" in this cage, and leave all the cages independent, plus the current relationship.

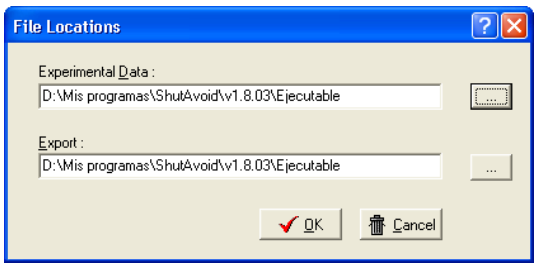


If any of the cages configured as Master or Slave was not detected at start-up of the application (or via the Setup option - Communications - Find boxes) or was discarded by the user via the Setup option - Checking box, row corresponding to the related cage displays the text "Not present" or "Discarded" (as applicable) next to the cage No related (column Related Box).

If any of the cages set in the yoked procedure was not detected at the start of the application, its related will have the role of "Independent". If during the screening process is a new cage, it also has the role of "Independent" by default.

5.4. Defining paths

Selecting the "User paths" option of the "Setup" menu, a window is open in which the Data and Export paths can be defined. The [...] buttons can be used for selecting a path from the directory tree.



6. Running an experiment

6.1. Introduction

When running an experiment, it is necessary to remember that two elements must join: the experimental subject and the schedule to be carried out by the experimental subject in the present session. So, when running an experiment, data recording is always done from one specific experimental subject under the control of a specific schedule. This is an experimental session.

The program makes no presumption about the real history of the experimental subject, leaving the control of the experiment entirely in the hands of the experimenter; the program is limited in controlling the carrying out of an experimental session by using a schedule selected by the experimenter.

SHUTAVOID for Windows operates based on the "active" shuttle boxes, understanding these to be those for whom a set-up of identification variables of the experimental subject has been defined.

An important characteristic of SHUTAVOID for Windows is to work with each box (role independent) or each yoked group (roles master-slave) independently. That is to say that each one is controlled independently, from the point of view of timing and from the point of view of the associated schedule.

6.2. The running window

When the "Run" option is selected from the main menu, a window like those shown in the next page is presented. This window controls the development of an experimental session in the Shuttle Box. The different parts are:

1. The title line shows the current box number.
   - If the role box is independent, only show the box number:

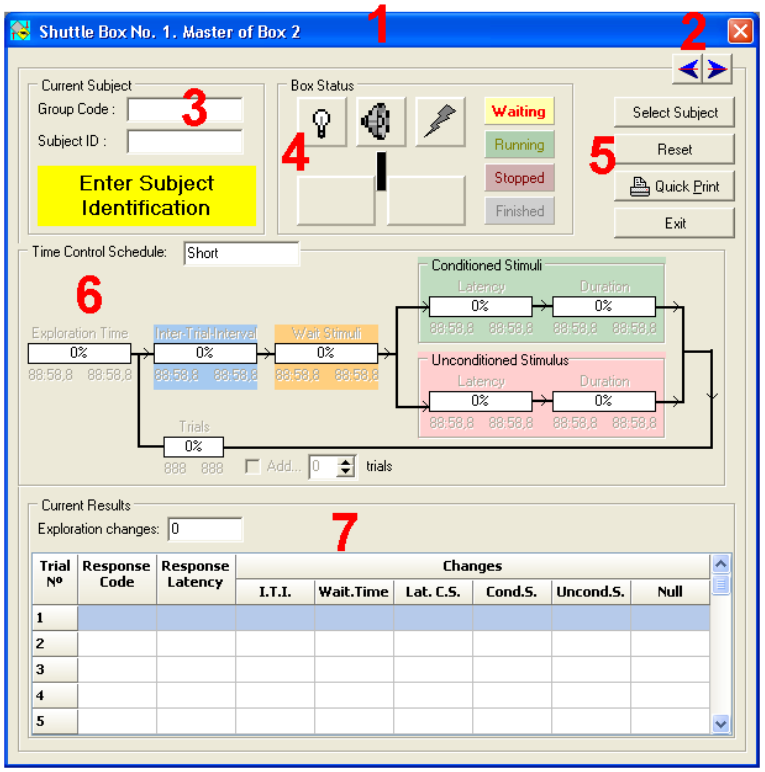
   - If the role of the box is master or slave, show its box number, its role and the related box number:

2. Right/left arrow buttons (top right).

   By pressing it is possible to access the rest of the active box, (those physically present; see [5.1. Checking Box]).

3. Subject identification

   It shows the identification of the subject. It also shows a yellow panel messages to the user, to indicate that you do in this cage.



4. Box status

   It shows a graphical representation of the state of the cage where the subject, the stimuli it receives and if the door is open or closed. In color panels the current state of evolution

of protocols in the cage is also shown. The status appears much brighter and intermittent.

5. Control buttons

With these buttons will control the implementation of protocols in the cage:

- First button: The text of the first button changes depending on the status of implementation of the protocol:
- [Select Subject]: pressing it will enter into a dialogue to identify subject, protocol and data file of the current cage.
- [Start]: When pressed, it waits until the subject is in the cage to start immediately the carry out of the protocol.
- [Stop]: Pause of the protocol in execution.
- [Continue]: Resumes execution of the protocol previously paused.
- [Finish]: After the execution of the protocol is expected to withdraw the subject of the cage or press to proceed with the data saved the experimental session that has just been carried out.

- [Reset] Terminate the execution of the current protocol, asking the user.

- [Quit Print]: Print the results there so far. These results appear in the "Current Results" from this screen.

- [Exit]: Allows you to exit this screen. If there are cages in progress, ask for confirmation.

6. Time Schedule Control

Displays the selected protocol and the evolution of the test is running in the cage. It also allows trials to add to the current protocol.

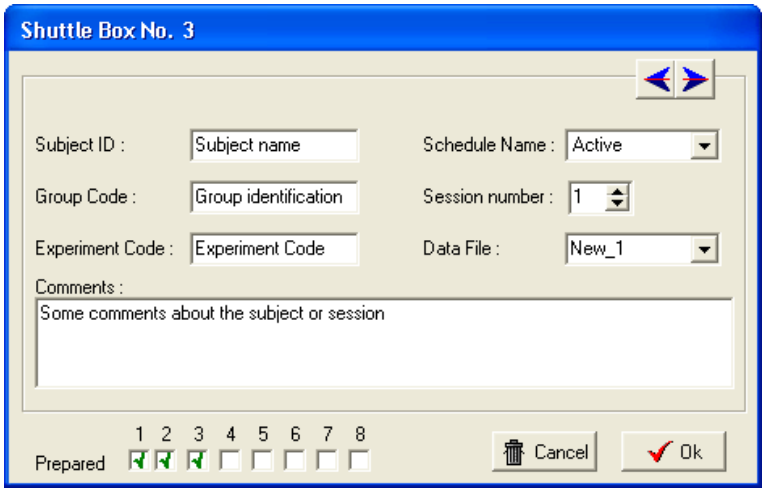
7. Current Results

On show the result of the test is running.

6.3. Carrying out an experimental session

6.3.1. Identifying the experimental subject

When [Select Subject] button is pressed, a window like the shown below appears.



Using this window, the user can enter all the information relative to the experiment to be developed in the current box. Data corresponding to the subject ID (name, group, experiment and comments) are meaning only for the user and serve as headers in data files.

Data corresponding to the schedule, the session number and the data file are indispensable for the software:

- Schedule name

  Is an unfolding list (to unfold, press the down arrow button at the right extreme), containing the name of all the available schedules (as defined in the corresponding section of the program). Between them, the user should select the schedule to be used in the current session with the current subject. For so yoked cage, the cage only master you can choose the protocol. In the slave cage on shown but cannot be changed.

- Session number

  It is a number that corresponds to the index of the current session for this experimental subject. Even not being an indispensable data, it is very important for the user to be aware of this, as it will allow the correct identification of the sequential order of data coming from different sessions with the same experimental subject.

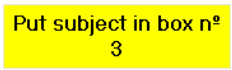
- Data file

  Allow to select of a file name to be used for the storage of data generated in the current session. This file is located in the folder Experiment data set in File Locations window. If an existent file name is used, data will be appended at the end of the file. Is the user who should decide the structure of filing (a

file per subject, group, session, etc.), keeping in mind that several shuttles using the same time could not share a file.

6.3.2. Entering the experimental subject

Once the subject ID has been entered in a given box, the text in the button changes to "*Start*" and the rest of data sections (Current subject, Time control) are updated according to the information entered in the subject ID window.



Pressing this button informs the experimenter to put the subject into the cage. For cages so yoked, the displayed message refers to this cage and its related.

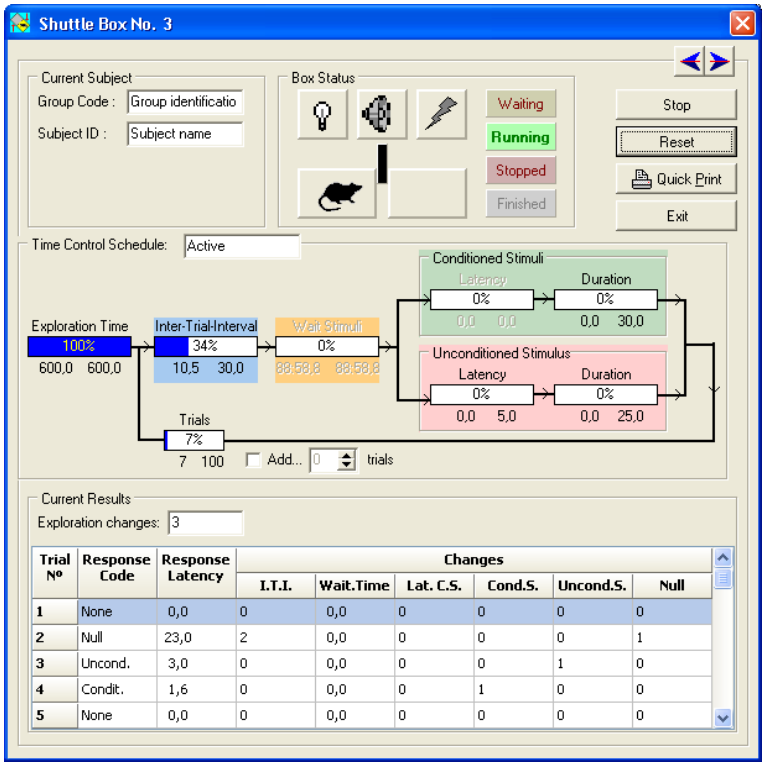
6.3.3. Running the session

Once the experimental subject is entered into the corresponding box or the two subjects in yoked mode, several changes occur, as it is shown by the figure in the next page:

- The shuttle box detects the experimental subject and one of the red LED's in the front panel shines, signaling the position of the experimental subject into the cage.
- The Box Status section of the window is updated accordingly, showing the rat in the corresponding panel.
- The Time control section shows the timing of the schedule, with the current time shown on the left side and with the percentile development show by the bars.
- The "Select Subject" button changes to "Stop".
- The "Waiting" button is turned off and the "Running" button begins to shine, indicating that the session is on.

Each time a trial is completed, the corresponding row in the "Current Results" section is updated, indicating the type of response observed ("R. Code"), their latency ("Res. Latency") and the period during which the response was obtained. In yoked mode, the response observed in the slave box always is empty and the changes of side are recorded in the column corresponding at the time in with happened.

Example for active avoidance paradigm:



Example for passive avoidance paradigm:

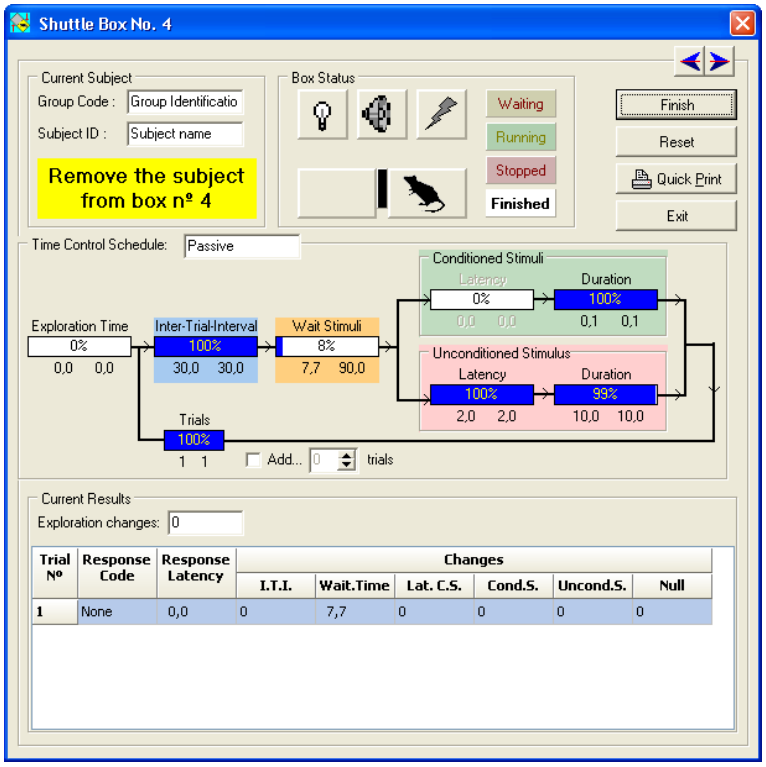


Table I show the way in which the response type is determined.

Table I. Types of responses, according to the moment in which they are detected.	
Type of response	Moment in which they are detected
Anticipation	The response has been produced during the intertrial interval. It could be that these responses are impossible, as occurs when the experimenter has selected, in the experimental Schedule, that trial RESET does not exist if a response is produced during this period.
Conditioned or avoidance	The response has occurred during the presentation of the conditioned stimulus, but before beginning the presentation of the unconditioned stimulus
Unconditioned or escape	Response produced during shock presentation but before finishing the latency response period defined in the experimental Schedule.
Null response	Response produced during the presentation of the unconditioned stimulus, but once the period of null latency responses established in the Schedule has been finalized. It can happen that these responses are impossible, as occurs when the experimenter has established that the null response latency is the same as the duration of the unconditioned stimulus.
Wait Stimuli	No response has occurred during the "Wait stimuli" and it is configured with a Length max.
Latency Conditioned Stimuli	The response has been produced during the "Latency conditioned Stimuli" and it is configured with a "Reset if answer".
None	No response has occurred during all the trial.

In the passive avoidance paradigm, the latency of entry to the black compartment is given in the column Wait T.

In any case, the program registers, independently, the changes in position and the responses and gives information about the moment in which each one has occurred.

6.4. Stopping a trial

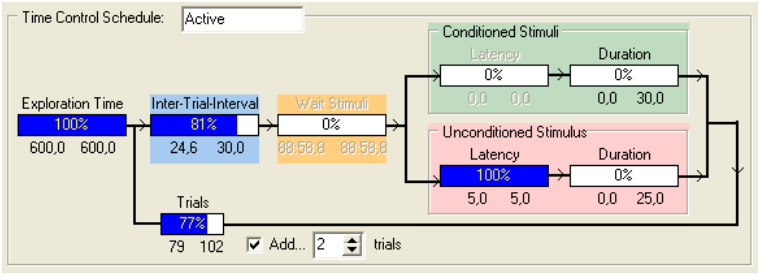
Once a session has begun it is possible to stop it as often as wanted (to resolve unseen problems that can affect it), by pressing the "Stop" button. However, from the timing control, considered as part of the Schedule, in a way that the session is restarted at the same point at which it was interrupted, independently of the length of the interruption.

The interruption of the work only affects the working box (and their related, in yoked mode); the remaining active boxes (if there are any) continue the session without being affected.

It is possible to opt for resume by pressing the same button (now with the text "Continue") again or aborting the work definitively (by pressing the "Reset" button), with a box whose work is interrupted. In the latter case, the session is not finished and the results obtained only correspond to the part of the session that has been completed.

6.5. Changing the number of trials forming a session

At any time during the development of an experimental session, new trials can be added in an independent or master box (the new trials will be appended at the end of the session).



[Add...] check-button allows enabling the new trials counter in which the number of trials to be added must be selected. New trials will be added to the sessions, as they were part of the current schedule. Nevertheless, the schedule definition will be not modified accordingly. So, the modification in the number of trials forming the session will affect only the current session and not any other being carried out with the same schedule. Newly added trials are marked with an "a" in the trial column of the "Current results" window.

New trials can be added at any time before a session is definitively over (when the experimental subject is removed from the shuttle box).

At the same time a number of trials are added to the session, the user can also exclude the results obtained in selected trials from the data to be evaluated.

Example of result table for active avoidance test:

Current Results

Exploration changes: 3

Trial Nº	Response Code	Response Latency	Changes					
			I.T.I.	Wait.Time	Lat. C.S.	Cond.S.	Uncond.S.	Null
1	None	0,0	0	0,0	0	0	0	0
2	Null	23,0	2	0,0	0	0	0	1
3	Uncond.	3,0	0	0,0	0	0	1	0
4	Condit.	1,6	0	0,0	0	1	0	0
5	None	0,0	0	0,0	0	0	0	0

Example of result table for passive avoidance test:

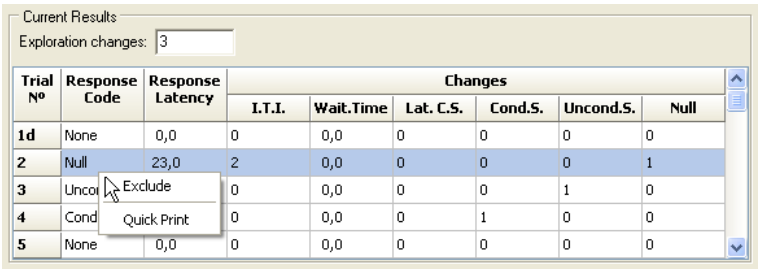
Current Results

Exploration changes: 0

Trial Nº	Response Code	Response Latency	Changes					
			I.T.I.	Wait.Time	Lat. C.S.	Cond.S.	Uncond.S.	Null
1	None	0,0	0	7,7	0	0	0	0

A trial is excluded by clicking with the left button of the mouse upon the corresponding line in the "Current results" window and

opening the contextual menu by clicking the right button of the mouse. This operation can be reverted as many times as wanted just repeating the same procedure. A "d" indicates the trial is marked as deleted.

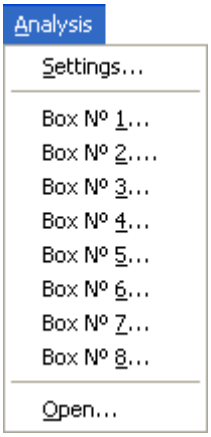


Data corresponding to an excluded trial is not eliminated from data files. When analyzing data, the excluded trials are included in the printouts but not considered in the numeric calculations.

6.6. End of Session

Once all the trials programmed in an experimental Schedule in an active box are finished, the results obtained are filed in the working drive & directory. The current box will be no longer available (this is indicated by the expression "*Finish*" in the column corresponding to State), unless the subject is removed of the box or the "Finish" button pressed. In yoked mode, the subjects of both boxes (master & slave) must be removed.

Data files can be recognized by the extension "scd" and it cannot be read nor modified directly. By using the options provided in the "Analysis" option, it is possible to re-open data files and generate ASCII-coded files with results from sessions as many times as necessary.

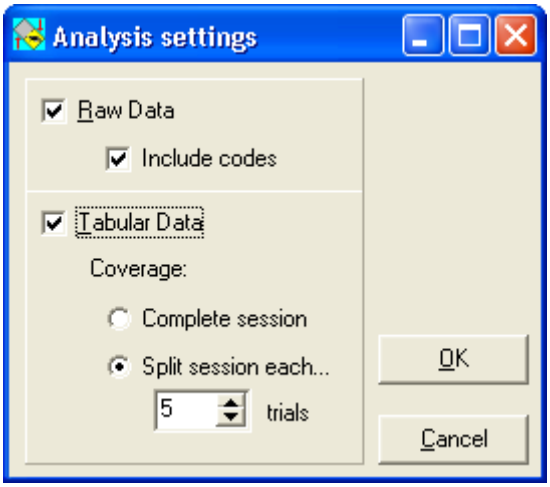


7. The Analysis window

At any time, data of the current or any other session can be acceded by selecting the "Analysis" option. When selected, a menu allows for the selection either of data from one of the active shuttle boxes or from any other data files. A "Settings" option allows for the configuration of the analysis to be carried out.

7.1. Analysis settings

When this option is selected, a window like those shown below is presented.



- Raw data

  This option forces the analysis to include raw data as it is described in section 6.2 of this manual. When the "Include codes" option is also checked, the raw data file will also include a list of the meaning of the codes used by these files to store the different events.

- Tabular data

  This option forces the analysis to include tabular data, as described in the section 6.2 in this manual. The coverage allows for the selection of the way in which data is totaled. If "Complete session" is selected, tabular data will present a list of all the trials and a final summary at the end of the session. If the "Split session each..." is selected, tabular data will be presented as described on "Complete session", but partial summary will be also generated at each "n" trials ("n" being the number selected at the spin edit box).

The output obtained in the remaining options of the analysis will depend on the selection carried out in the "Analysis/Settings".

7.2. Raw data files

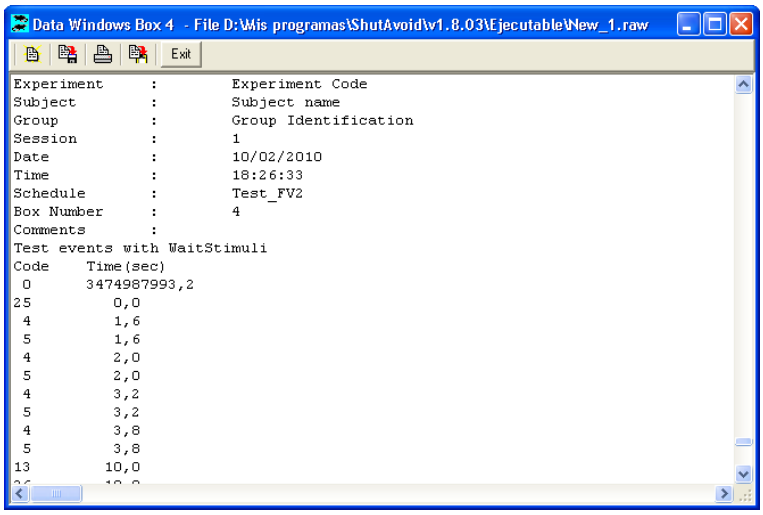
These files, which are recognized by their "RAW" extension, contain sequentially codified information about ALL the events produced throughout the development of the experimental session and the time of occurrence of each one. The code used for recording events is that reflected in Table II.

Table II. Code of events used by the **SHUTAVOID for Windows** program in the "RAW" original data files

Code	Description
0	Session begins
1	ITI begins
2	Conditioned stimulus begins
3	Unconditioned stimulus begins
4	Change of position of experimental subject
5	Emission of valid response
7	ITI ends
9	Unconditioned stimulus ends
10	Interruption begins
11	Interruption ends
12	End of experimental session
13	Exploration ends
15	Wait Stimuli begins
16	Wait Stimuli ends
23	Latency conditioned stimuli begin
24	Latency conditioned stimuli end
25	Exploration begins
26	Trial begins
27	Trial ends
28	Conditioned stimuli begin
29	Conditioned stimuli end

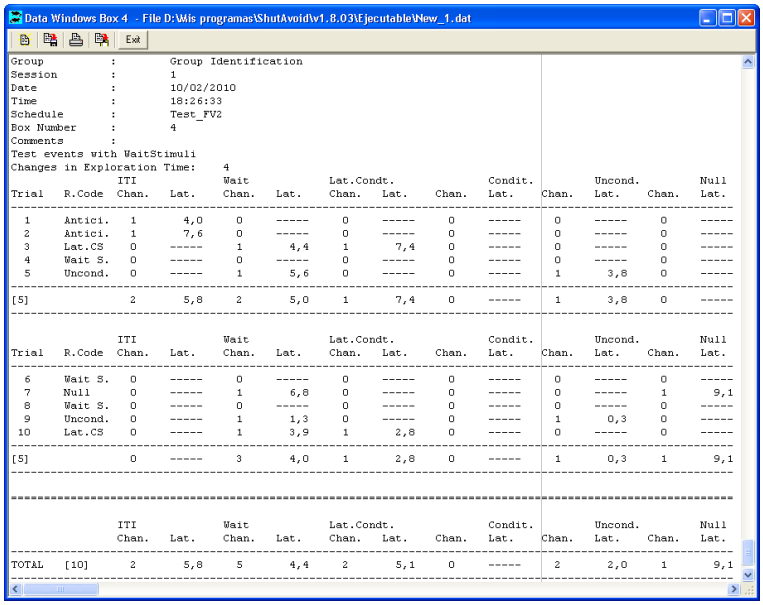
30	Latency unconditioned stimuli begin
31	Latency unconditioned stimuli end

The aim of these files is, on the one hand, to permit data access, making other forms of analysis distinct to those given by the program possible. On the other hand, these files permit the checking of the correct operation of the program, because in them the beginning and ending of each of the different periods that make up the trials are reflected. A sample of the aspect of the data window with raw data is shown below.



7.3. Tabular data files

Apart from the data contained in the file already mentioned in the previous section, the program elaborates an additional file, in which the information for each trial that forms the experimental session is summarized.



This file contains a heading in which the data about the identification of the experimental subject, just as they were typed when the experiment was carried out, the date and hour and the name of the experimental Schedule under which the session was developed is included.

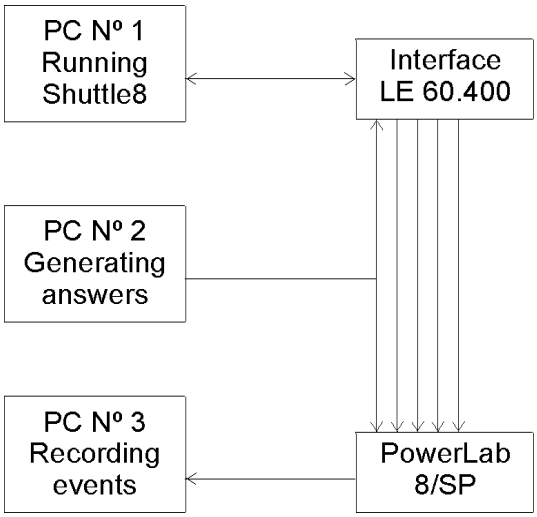
After that, as many data lines as trials in the experimental session are presented. Each of these lines contains the information specified in Table III (see figure 8).

Table III. Information contained in the ".DAT" results file.	
Column code	Contents
Trial	Current trial number
R.Code	Code of the type of response recorded (see table I)
ITI Chan.	The number of changes of position detected during the inter trial intervals.
ITI Lat.	Response latency (in seconds) in the inter trial intervals
Wait Chan.	The number of changes of position detected during the Wait Stimuli.

Wait Lat.	Time of the first change of position in the Wait Stimuli period.
Lat.Condt. Chan.	The number of changes of position detected during the Latency Conditioned Stimuli.
Lat.Condt. Lat.	Time of the first change of position in the latency of conditioned stimuli period.
Condt. Chan.	The number of changes of position detected during the presentation of the conditioned stimulus and before the presentation of the unconditioned stimulus.
Condt. Lat.	Time of the first change of position in the conditioned stimuli period.
Uncond Chan.	The number of changes of position recorded during shock presentation and before finishing the latency of null responses.
Uncond Lat.	Time of the first change of position in the unconditioned stimuli period.
Null Chan.	The number of changes of position detected during the presentation of the unconditioned stimulus but after the null responses latency has finished.
Null Lat.	Time of the first change of position detected during the presentation of the unconditioned stimulus but after the null responses latency has finished.

8. Appendix A – Validation procedure

SHUTAVOID has been checked for the accuracy in the timing of the different parameters as well as in the detection of the external signals (mainly the change in the position of the experimental subject within the shuttle box).



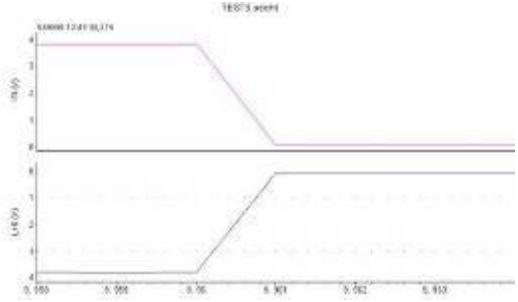
The following is the structure of the validation set-up:

Three PC computers are interconnected as the diagram shows it. Computer 1 is running SHUTAVOID and thus generates output according to a pre-defined protocol. The output from the program is addressed to the LE 60.400 interface and computer 3 picks it up. In computer 2 a specially tailored program generates "answers" at defined intervals. The answers mimic those produced by the experimental subject when moves from one side to the other of the shuttle box. Responses are presented in the LE 60.400 interface (to be detected by SHUTAVOID); SHUTAVOID generates a signal, also sends through the interface to the computer 3, when the response is detected. Responses generated in computer 2 are also sent to the computer 3.

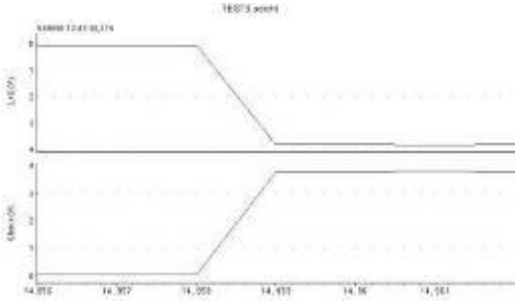
Computer 3 records, using a PowerLab 8/SP (from ADInstruments, Sydney) run with Chart, the output from the LE 60.400 interface and from the computer 2. Each output line in the interface is addressed to a different input channel in PowerLab, thus allowing studying the duration of each one of them and the delays in changing the status of the outputs (light ON/OFF, sound ON/OFF, shock ON/OFF). As PowerLab also receives the answer when generated by computer 2 and when detected by SHUTAVOID, the delay of SHUTAVOID in detecting the response and in "react" according to the schedule can be also studied.

Timing accuracy

The following graph shows the transition between the end of the ITI (upper) and the beginning of the presentation of Light + sound (lower, inverted logic).

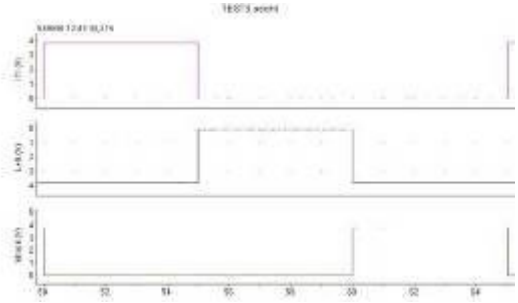


Note the synchronization between the offset of the ITI and the onset of CS. The transition time is 0.001 sec.

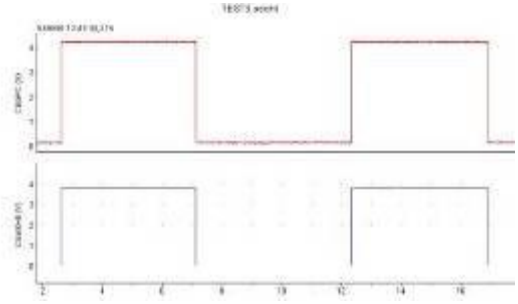


The graph above shows the offset of the CS (upper) and the onset of the UCS (lower). Again, both are perfectly synchronous and with a transition time of 0.001 sec.

The following graph shows the onset and offset of one ITI, and the CS and UCS presentation.



Programmed duration is 5 sec each, exactly the values obtained from the graph.

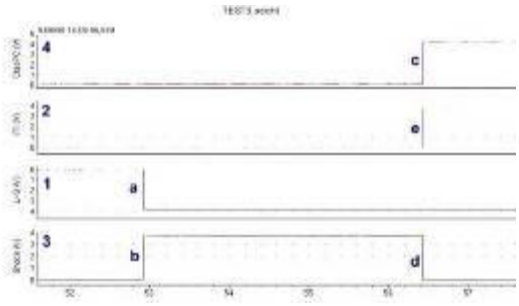


The graph above shows the "answers" generation in PC2 (upper) and the detection by SHUTAVOID (below). There is an exact

synchronism between both, showing that the delay in detecting external signal is almost negligible.

In the next graph, a partial trial is shown. At the beginning of the chart, we can see that the CS is being presented (trace nº1), as the previous ITI was over (trace nº 3) and the UCS is off (trace nº 2). The subject answers should appear at the trace nº 4.

After some seconds (the CS is shown only partially), the CS presentation ends (SHUTAVOID achieves the programmed duration, see a). Immediately, begins the presentation of the UCS (at b). About 3.5 seconds after the beginning of the UCS presentation, a response occurs (see c). This makes the UCS stop (see d) and a new trial to begin (as it is shown by the beginning of a new ITI (see e).



The resulting data file, with ms-precision, is compared with the corresponding "raw" file generated by SHUTAVOID, looking for the coherence between data in this file and real occurrence of events, as recorded in the external data file.

From this analysis we have seen that timing of events with SHUTAVOID (Windows version) is very accurate; the difference between programmed and actual time-period is always less than 10 ms.

9. 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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