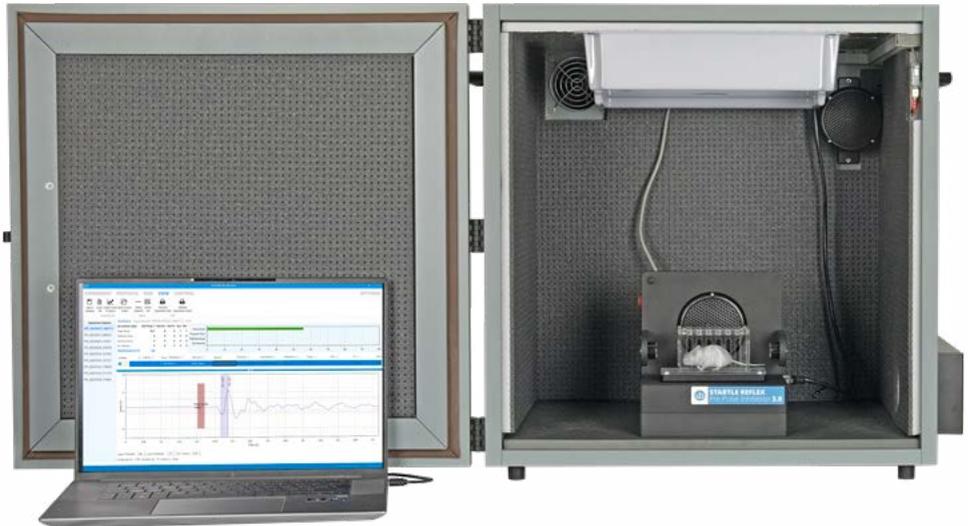# Pre-Pulse Inhibition of Startle Reflex (PPI)
User Guide
Rev. 3.1 - November 2025

ugobasile.com

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YOUR COMPANION IN
DISCOVERY SINCE **1963**

SAFETY CONSIDERATIONS

Although this instrument has been designed with international safety standards, it contains information, cautions and warnings which must be followed to ensure safe operation and to retain the instrument in safe conditions.

Service and adjustments should be carried out by qualified personnel, authorized by Ugo Basile organization.

Any adjustment, maintenance and repair of the powered instrument should be avoided as much as possible and, when inevitable, should be carried out by a skilled person who is aware of the hazard involved.

Capacitors inside the instrument may still be charged even if the instrument has been disconnected from its source of supply.

CE CONFORMITY STATEMENT

Manufacturer	**UGO BASILE srl**
Address	Via G. di Vittorio, 2 – 21036 Gemonio, VA, ITALY
Phone n.	+39 0332 744574
Fax n.	+39 0332 745488

We hereby declare that

Instrument:	**Startle/PPI System**
Catalog numbers:	**48101, 48101-2, 48101-3, 48101-4**

is manufactured in compliance with the following European Union Directives and relevant harmonized standards

- *2014/35/UE relating to electrical equipment designed for use within certain voltage limits*
- *2014/30/UE relating to electromagnetic compatibility*
- *2011/65/UE and 2015/863/UE on the restriction of the use of certain hazardous substances in electrical and electronic equipment*

Account *Manager*

Mauro Uboldi

Nome / *Name*

June 2025

Date

Firma / *Signature*

Table of Contents

1. Product features and general information

One of the major challenges in Neuroscience is reproducing core symptoms of complex diseases, such as schizophrenia working with laboratory animals.

However, pre-pulse inhibition of the startle reflex represents an effective way to solve this challenge and has been used by scientists worldwide with great success, if one considers the more than 10,000 papers published using this methodology

Startle-PPI devices allow the quantification of the pre-pulse inhibition (PPI) of the startle reflex.

The startle magnitude elicited by an intense stimulus (auditory) is reduced when it is preceded by a weaker pre-pulse stimulus.

Impaired PPI is observed in schizophrenia (Braff et al. 2001, Swerdlow et al. 2008), Tourette's syndrome (Swerdlow et al. 2001), Huntington disease (Swerdlow et al 2005), Bipolar disorder (Perry et al. 2001).

Available configurations are 1, 2, 3 or a maximum of 4 Startle-PPI cages

The Ugo Basile system is fully automated both for the preparation of the procedure and for the analysis of data.

Main features include:

- Automatic detection of Startle Reflex in mice and rats.
- A typical system combines the following elements:
  - Dedicated Software;
  - Up to 4 Isolation Cubicles that includes an infrared (I.R.) light, a visible light, a loudspeaker and a noiseless fan, all conveniently positioned inside the sound attenuating box
  - Stimulating/Recording (S/R) Platform that includes the flash light (only for the mouse version), speaker and detection system

Different animal holders to match animal dimensions.

Flexible testing, easy to set up. Simply define trial number, sound and light stimulus and timing of experimental sequences (all fully randomizable): Pulse, Pre-pulse, Inter-Pulse Interval, Inter-Stimulus Interval, TTL Outputs to control external devices (with optional I/O Box)

The Ugo Basile system is fully automated, both for the preparation of the procedure and for the analysis of data.

The new version 3 of Startle/PPI system has been recently upgraded to provide enhanced precision, flexibility, and ease of use through:

- Easy Platform Calibration: The sensitivity and offset of the load cell can now be calibrated automatically using the included calibration tool
- Easy to swap between PPI and Fear Conditioning: Thanks to the new CBOARD01 board, speakers for different modules are managed

independently. No manual unplugging is needed. Switching from PPI to FC or vice-versa now only requires changing a Fear Conditioning grid cage with a PPI platform. the use of a camera is essential for freezing detection but is optional in PPI systems

- Optimized Auxiliary TTL Signals: The AUX1 (linked to stimuli) and AUX2 (for environmental controls) signals are now accessible via an optional TTL I/O Interface, offering a cleaner and more customizable setup tailored to the experimental protocol.

The latest software version includes:

- Calibration of platform sensitivity directly via firmware/software
- Fine-tuning of response thresholds.
- Resizeable graph in the VIEW page.
- User-defined cubicle numbering (1, 2, 3, or 4), no longer dependent on the operating system.

2. What's in the box

SKU: 48101, 48101-2, 48101-3, 48101-4

In the Startle PPI package You will find the following items depending of the order you placed.

- 1 Standard isolation cubicle (or 2 or 3 or 4 units depending the order)
- 1 Startle PPI platform (rat or mice depending the order)
- X Animal cage (restrainer) depending the order 3 for mice or the one you ordered for rats
- 1 Power supply power cords (according to your country layout)
- 1 USB cable 5m
- 4 m transparent pipe (only if Air-Puff option included)
- 1 USB memory key, which contain the Startle PPI software + the quality control certificate and the instruction and assembling manuals
- Cubicle inside IR/visible B/N USB Video camera, depending the order

Optional items for multiple cubicle only (2 or 3 or 4 units)
- 1 Startle PPI calibrator unit
- 1 Ugo Basile 4-ports industrial USB Hub
- 1 USB cable for the HUB 2 Mt.
- 1 External USB Hub power supply

3. General

Pre-pulse Inhibition of startle reflex (PPI) is an experimental paradigm commonly used in rodents, such as mice and rats, to study sensorimotor gating, which refers to the brain's ability to filter out irrelevant sensory stimuli and prevent them from overwhelming the processing of more important stimuli.

Sensorimotor gating refers to the ability of a sensory event to suppress or decrease a motor response, in this case, a startle response

The Startle PPI paradigm has been widely used in neuroscience research to study sensorimotor gating deficits in different animal models, including genetic mouse models and those induced by pharmacological agents. It has also been employed to investigate the neural mechanisms underlying sensorimotor gating and to evaluate the effects of potential therapeutic interventions for disorders like schizophrenia, where PPI deficits are often observed.

A typical experiment includes a startle baseline measurement as a response to a loud sound (more rarely to a light or air-puff). A pre-pulse stimulus is delivered shortly before the loud stimulus and is supposed to decrease the startle reaction in healthy animals. Normally the time between trials is randomized to ensure that the animal response is not influenced by previous stimuli.

Researchers can manipulate various experimental parameters to investigate the factors influencing PPI, such as the intensity of the pre-pulse, the interval between the pre-pulse and startle stimulus (known as the prepulse-to-pulse interval or IPI Inter-Pulse-Interval), and the genetic or pharmacological manipulations of the animals.

4. Instrument description

The Ugo Basile Startle-PPI system is based on the light (mice only), sound or air-puff (optional) pre-pulse and pulse stimulations.

Platform and restrainer are positioned within an isolation cubicle to avoid external interferences and cross talk among cages (maximum 4 cages connected to 1 PC).

Settings, protocol configuration, experiment run, device management and results, are all controlled and displayed by a specific included software.

Pulses and pre-pulses consist of white noise and frequency modulated sounds, lights or air-puffs.

The animal is restrained in a Plexiglass tube/box sitting on a force sensor. The dedicated software analyses data automatically, as startle response amplitude and %PPI.

The various procedures expose the animals to "Trials" of different nature:

- Pulse alone trial: only the strong stimulus is presented
- Pre-pulse alone trial: only the weak stimulus is presented
- Pre-pulse-pulse trial: the strong stimulus is preceded by the weak (pre-pulse) stimulus
- No-stim trials: no stimulus is delivered but the response of the animal is recorded to serve as a baseline

Experimental parameters can all be adjusted, linked, spaced in time, etc., to virtually build in a seamless and user-friendly manner any type of startle and startle/PPI protocols.

5. Installation

5.1. Unpacking & preliminary check

Check the contents of the shipment for completeness, check list to hand, and visually inspect the instrument as soon you take it out the packaging.

If the instrument is damaged, immediately inform the shipping agent or carrier and notifying us by email at support@ugobasile.com.

Inspect the instrument for damages such as scratches, broken or loose parts. If after having tested it, the instrument fails to meet rated performances, contact our company immediately via email at service@ugobasile.com.

Notes on the User Guide

This User Guide can be found on the provided USB Key.
We recommend to read thoroughly this manual, as it contains essential information for the correct installation and operation of the instrument.

Please save the manual in a safe place, ready to be consulted by qualified personnel who use the instrument. Print it only if necessary.

Our User Guide are available as free download on our web site. For any additional information and/or assistance, you are welcome to contact our Service Department at service@ugobasile.com, specifying the serial number of the instrument.

5.2. Safety instruction

To ensure safe operation DO NOT attempt to open or perform any service work before having contacted Ugo Basile support team.

Pay attention while this device can emit a very loud sound at low and high frequencies that can damage human and animal auricle; always close the sound isolation cubicle door before running the test.

5.3. Intended Use

The Startle PPI is intended for
Investigation use on laboratory animal only.
DO NOT USE ON HUMANS.

5.4. Additional Safety Consideration

1. Use original accessories and spare parts only.
2. Immediately disconnect and replace damaged main cord.
3. Do not obstruct access to the power module.
4. Do not operate in hazardous environment or outside prescribed environmental limitation.
5. Do not spray any liquid on the connectors or on the electronic unit.

Ugo Basile cannot in any way and form be held responsible for damage caused to things and people and warranty will be void, due to:

- Incorrect electrical supply.
- Incorrect installation procedure.
- Incorrect or improper use or, in any case, not in accordance with the purpose for which the instrument has been designed and the warnings stated in the instruction manual supplied with the instrument.
- Replacement of original component, accessories or parts with others not made by Ugo Basile.
- Servicing carried out by unauthorized personnel.

5.5. Assembling the instrument

Place the isolation cubicle on a flat surface (usually on a desk) pay attention to leave a minimum of 15 cm space around the rear, the left and the right side of it, to avoid air obstruction.
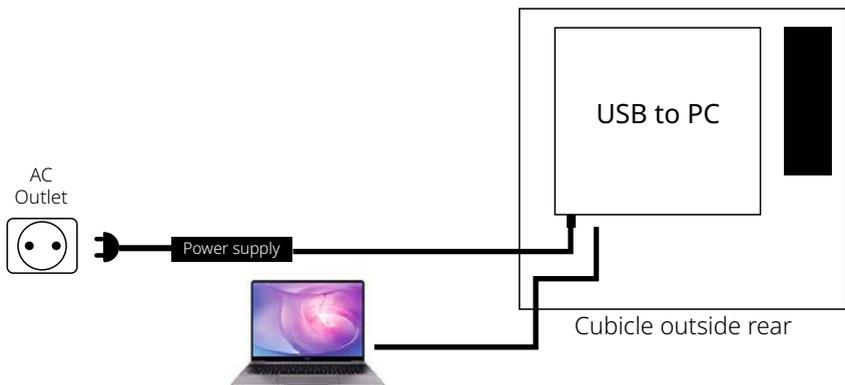
The surface must be stable and should not propagate, from the table or the floor.

Place the Startle PPI cage inside the isolation cubicle in the centre of the floor.

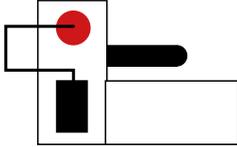Make the correct electric and pneumatic connection according to the following diagram:

Refer to the 48101 Startle PPI assembling manual v2.2 Nov 2025.pdf for the connection diagram for multiple cubicle.
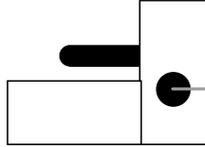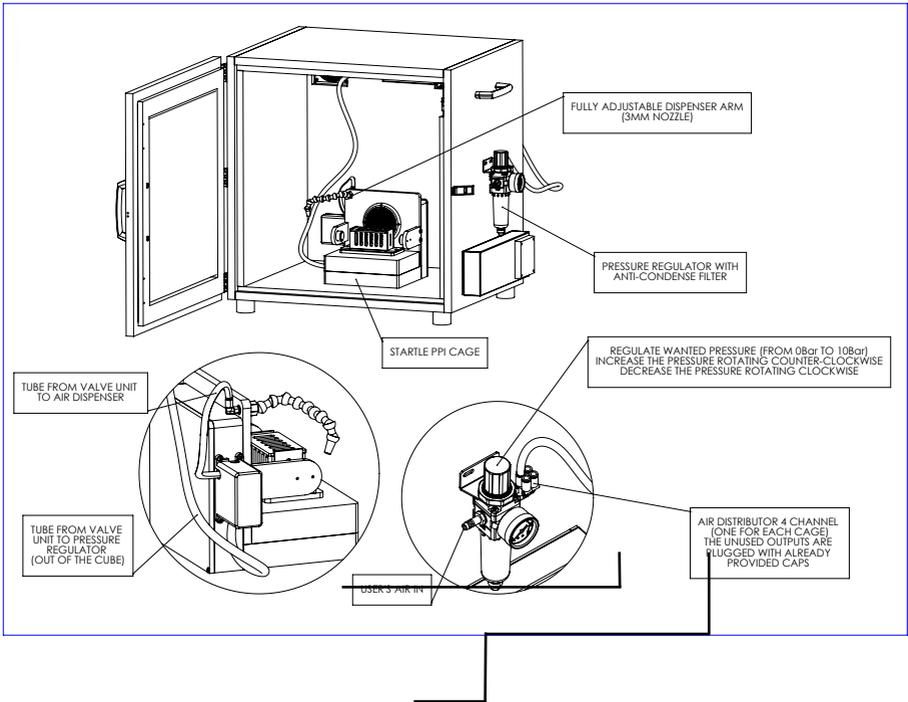
Startle PPI Connection diagram

Cubicle inside

CABLE RJ50 already in the cubicle

Startle PPI Platform left view

Startle PPI right view

FULLY ADJUSTABLE DISPENSER ARM (3MM NOZZLE)

PRESSURE REGULATOR WITH ANTI-CONDENSE FILTER

STARTLE PPI CAGE

REGULATE WANTED PRESSURE (FROM 0Bar TO 10Bar) INCREASE THE PRESSURE ROTATING COUNTER-CLOCKWISE DECREASE THE PRESSURE ROTATING CLOCKWISE

TUBE FROM VALVE UNIT TO AIR DISPENSER

TUBE FROM VALVE UNIT TO PRESSURE REGULATOR (OUT OF THE CUBE)

USER'S AIR IN

AIR DISTRIBUTOR 4 CHANNEL (ONE FOR EACH CAGE) THE UNUSED OUTPUTS ARE PLUGGED WITH ALREADY PROVIDED CAPS

Connection diagram of the Startle PPI system: on the rear external side of the cubicle there is a black box containing the electronic unit. From here, a USB cable connects the system to the PC and a power cable provides power.

5.6. Installing the Startle PPI software

Startle PPI software system requirements:

Feature	Minimum	Recommended
CPU	Intel i7	Intel i9
Memory (RAM)	8 GB	16 GB
Storage (free space)	1 GB	10 GB
Monitor	1024 x 768	1920 x 1080
Operating system	Microsoft Windows 10 64bit	Microsoft Windows 11 64bit
Keyboard	Yes	Yes
Mouse	Yes	Yes
USB port	1 free (USB 2.0)	1 free (USB 2.0) for each cage

You will find the software installation kit inside the provided USB memory key.

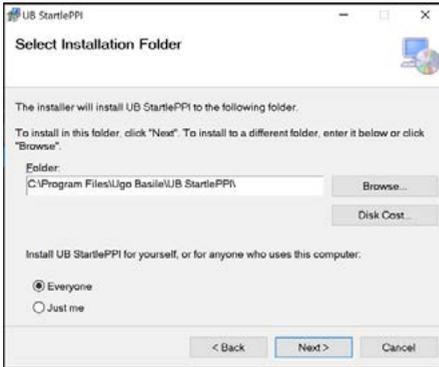The installation package contains two files:

setup.exe

StartlePPISetup.msi

Double click the setup.exe application to begin the install process. If an older version of the application is already installed it is overwritten with the new version. Data is not deleted by the setup process.
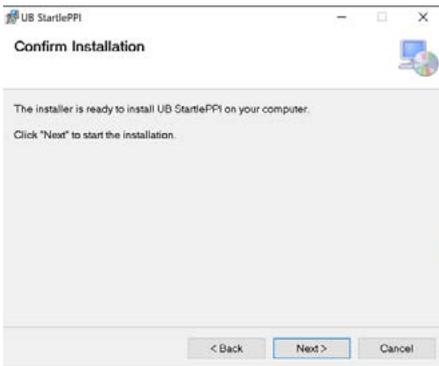
After starting the setup process this window appears:
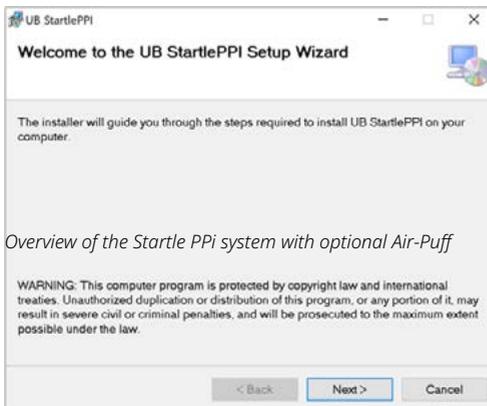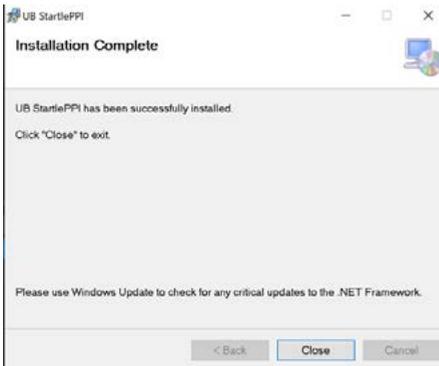
 Click Next

Here you can change the installation path. If more users share the same machine with different profiles, it is suggested to choose "Everyone".

Click Next

Click "Next" again to start installation process

When installation process ends, the following message is shown

A short-cut for starting the application is placed automatically in the Start menu and on the Desktop.

5.7. Starting the application

Before starting the application, ensure that the USB interface cable is connected to the available cubicles.

To start the application just double click the short-cut on the desktop. A splash screen appears for a few seconds while the application is loading.

The program, once started, automatically creates a "Startle-PPI" folder in your "Documents" folder (if not already present).

In this folder other sub-folders are created, containing the following files and folders:

DB: contains the program database with all data resulting from the experiments done. The default file is named "**db.sqlite**". If the file is deleted, a new empty file is created at the next program launch.

EXPERIMENTS: this folder contains all the created experiments in XML format

SCRIPTS: this folder contains the protocol scripts and the file extension is ".PPI"

EXPORT: this folder is used as the destination when exporting the experiments in CSV/XLSX format.

UPDATES: in this folder it is possible to eventually store firmware update files. (See paragraph "11.2. Firmware Update")

5.8. Using the application

General information

The application pages are identified by tabs:

The currently selected page is highlighted.

To close the application, click the icon in the top right corner.

On the bottom bar, the footer, you will find the current date and time and the program version.

6. Experiment Page

This is the screen where you can define the information of the treatments, stages and animals involved in the experiment.

First of all it is necessary to define one or more treatments.

Then you can define one or more stages.

Then you can add the animals. Animals can be added in groups. Each animal can be assigned to one treatment only.
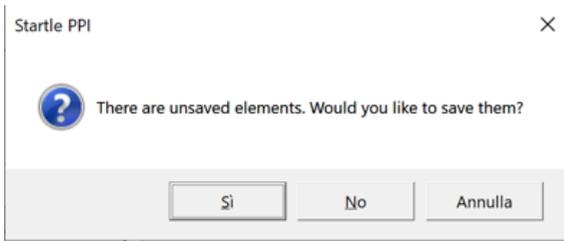
Adding treatments, stages (along with the number of trials for each stage) and animals, the right table is automatically populated.

The top menu offers these buttons:

6.1. New Experiment

It allows to clear all current data and start entering new data for an experiment. A name for the experiment is necessary. A warning appears if unsaved data is present:

Then a dialog window opens, letting entering a name for the experiment.
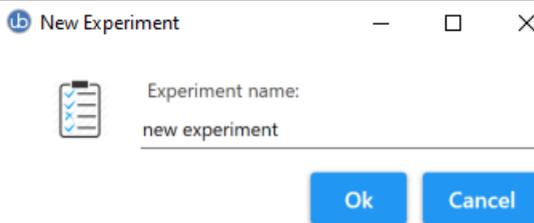
Type in a name for the experiment and hit "OK". The new experiment is now ready to be filled with treatments, stages and animals. One treatment and one stage are created by default.

6.2. Load experiment

This button is used to load a previously saved experiment.

6.3. Save experiment

This button is used to save the current experiment. If you want to create a new experiment, please give a different name to the file to prevent overwriting the existing one.

6.4. Print Experiment

This button prints the current "Trials" table (the one in the right table of this page).

6.5. Add new treatment

This button adds a treatment to the treatments table. Treatments are automatically named "Treatment 1, Treatment 2 and so on. You can rename any treatment clicking the name in the treatments table:

6.6. Remove Selected treatment

Il allows to remove the currently selected treatment. Only treatments with no assigned animals can be deleted.

6.7. Add New Stage

This button adds a stage to the stage table. Stages are automatically named "Stage 1, Stage 2 and so on. You can rename any stage clicking the name in the Stages table, same as explained before for the treatments. Moreover, here you can define how many times a stage must be repeated defining the value in the "# of Trials" column.

6.8. Remove Selected stage

Il allows to remove the currently selected stage.

6.9. Add new animals

When this button is pressed, a dialog window opens, letting you to enter one or more animals:

 Animals can be assigned to one only treatment. Animals are automatically named as Animal 1, Animal 2, and so on. Then, in the animals table, you can assign the desired Animal ID that uniquely identifies the animal.

6.10. Remove Selected animal

Il allows to remove the currently selected animal.

6.11. Trials table

When any change is done to the treatments, stages and animals, it is automatically reflected to the trials table.

Experiment name: **temporary**

Preview by # of cubicles: 1 ▼   Order by: Stage ▼

TRIALS (10)

| Run	Animal ID	Color	Gender	Weight	Treatment	Stage	Trial
1	IT123	White	Male	30	Saline	Stage 1	1
2	ID345	White	Male	30	Saline	Stage 1	1
3	Animal 5	White	Male	30	Saline	Stage 1	1
4	Animal 3	White	Male	30	Drug	Stage 1	1
5	Animal 4	White	Male	30	Drug	Stage 1	1
6	IT123	White	Male	30	Saline	Stage 1	2
7	ID345	White	Male	30	Saline	Stage 1	2
8	Animal 5	White	Male	30	Saline	Stage 1	2
9	Animal 3	White	Male	30	Drug	Stage 1	2
10	Animal 4	White	Male	30	Drug	Stage 1	2
11	IT123	White	Male	30	Saline	Stage 2	1
12	ID345	White	Male	30	Saline	Stage 2	1
13	Animal 5	White	Male	30	Saline	Stage 2	1
14	Animal 3	White	Male	30	Drug	Stage 2	1
15	Animal 4	White	Male	30	Drug	Stage 2	1

In this example there are two treatments (named "Treated" and "Untreated") and 2 stages of two trials each. We Added 4 animals, that are assigned two to each treatment.

The first column, named "Run" indicates the experiment subsequent run, as it depends on the number of animals being tested concurrently (so it depends on the number of cubicles you will use). The number of cubicles can be selected in the "Preview by # of cubicles" dropdown list:

Preview by # of cubicle: 1   ▲

You can choose from 1 to 4 cubicles.

Moreover you can choose in which order you wish to test the animals (by treatment, by stage of by animal):

Order by: Stage   ▲

	Stage
Gender	Weigh...	Treatment
Male	3	
Male	3	Animal
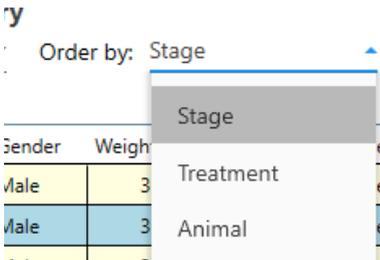
Regardless the chosen distribution mode and the number of cubicles used, in each run are assigned only animals that belong to the same treatment, stage and trial. So, in this example, no more than two animals can be tested at the same time.

7. Protocol Page

The protocol page allows to create a script for the experiment, where you define the environment characteristics and the stimuli sequence.
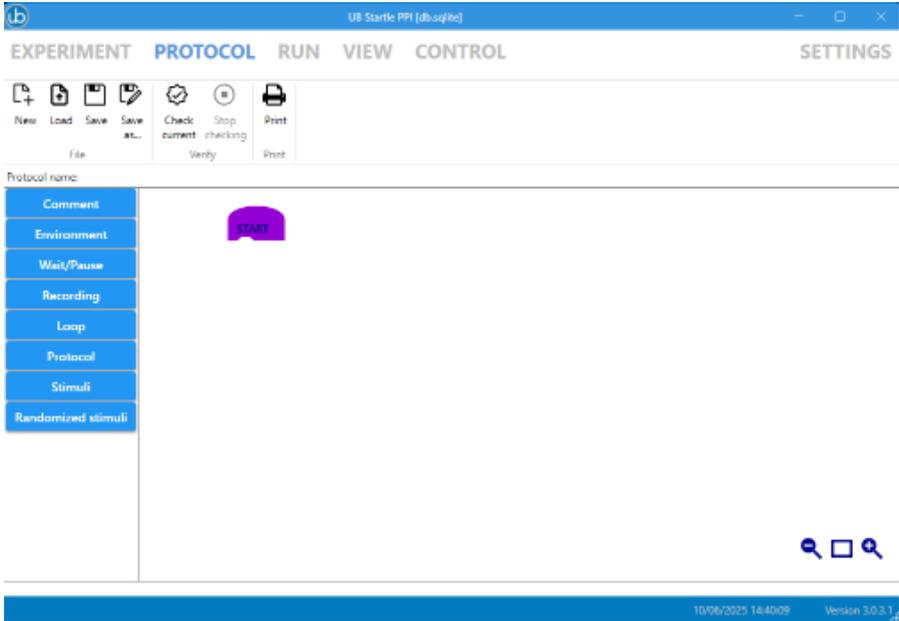
7.1. Protocol menu
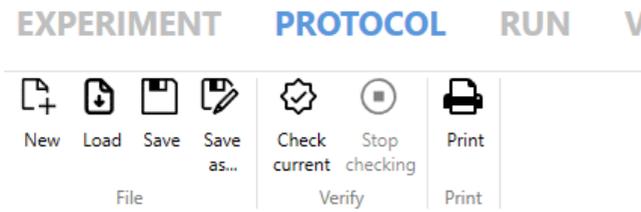
7.1.1. New File

This button allows to create a new protocol

7.1.2. Load File

Allows to load a previously saved protocol

7.1.3. Save File

Saves the current protocol with the same name

7.1.4. Save file as...

Lats you to save the current protocol giving a new name.

7.1.5. Check current

Checks the current protocol for errors or wrong values

7.1.6. Stop checking

Stops the current check

7.1.7. Print

Prints out the current protocol.

7.2. Protocol Blocks
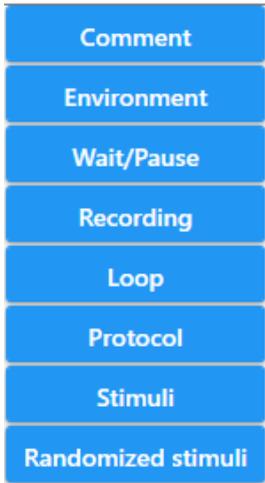
On the left side the block categories are listed and hereafter described. The blocks can be dragged and dropped after (or before) a block already present in the main area.

7.3. Comment

The **Comment block** (//) is used just as a remark to describe anything you want in the protocol. It does not make any action.

The **Step Name Block** lets you to enter a description that is then added to all the following stimuli, and it can be used to filter out data. For example, you can give names such as "Acclimation basal phase", "Pseudo random phase", "Verification basal phase" and so on.

7.3.1. Environment

White noise start lets you start a background white noise in the cubicle, defining the dB level from 45 to 120dB.

White noise stop disables the white noise output.

Fan Start starts the cubicle fan.

Fan Stop stops the cubicle fan.

Visible Light Start turns the visible light on defining the brightness from 10 to 100%.

Visible Light Stop turns the visible light off.

IR Light Start turns the IR light on defining the brightness from 10 to 100%.
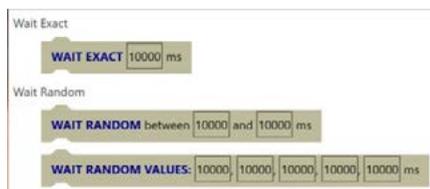
IR Light Stop turns the IR light off.

Aux (Out 2) Start turns the auxiliary output on.

Aux (Out 2) Stop turns the auxiliary output off

Stop All turns off all the environment outputs off.

7.3.2. Wait/Pause

Wait Exact adds a fixed delay. The value is in milliseconds, so a value of 10000ms corresponds to 10 seconds

Wait Random creates a random delay between the given limits

Wait random values peeks a delay between 5 given values

7.3.3. Recording

Recording Start: this block is used to define that the following stimuli will be recorded. Values for this block are described later.

Recording Stop: the following stimuli are not recorded.

The **START RECORDING** block allows to set the following values:
- **Capture window**: it indicates the time window that is recorded after the end of the stimulus.
- **Peak to peak**: it is the maximum time length of the startle reaction, from peak to peak. If the peaks time distance is greater than this value, the reaction will not be marked as a detection.
- **Pre-recording**: it is the time period that it is recorder prior to firing the stimulus. This can be useful to verify whether the animal was already moving in the cage before the stimulus is fired.
- **Detection time limit**: the startle reaction is searched from the stimulus start (or the beginning of the second pulse in the prepulse-pulse stimuli) until this time after the end of the stimulus.

The total time recorded for a stimulus is calculated with the sum of:
- Pre-recording time
- Pre-pulse duration (if it is a Prepulse-pulse stimulus)
- IPI, aka Inter-Pulse-Interval (if it is a Prepulse-pulse stimulus)
- Pulse duration
- Detection window

The startle event search is done:
- Pulse or Prepulse Alone stimuli: from the start of the pulse till the end of the pulse plus the detection time limit
- Prepulse-Pulse stimuli: from the start of the pulse till the end of the pulse plus the detection time limit

During
- the pre-recording
- the Prepulse and the IPI in the Prepulse-Pulse stimuli
- and after the Detection time limit

Any animal movement is recorded but not marked as a detection even if it goes beyond the threshold limits

7.3.4. Loop

Repetition Loop

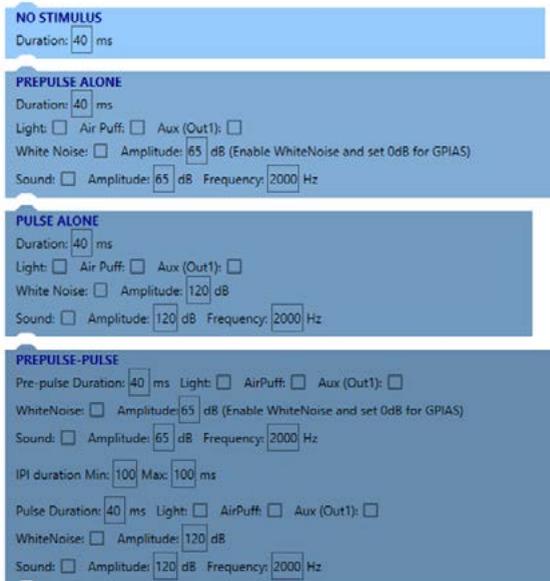**Repeat**: you can define a loop to repeat the same sequence a number of times.

7.3.5. Protocol End

Protocol

End Trial: this block must be set at the end of the protocol.

7.3.6. Stimuli

NO STIMULUS is used for auditing the animal movements when no any stimulus is fired. The stimulus reaction is recorded in the same way of the other stimuli.

PREPULSE ALONE and **PULSE ALONE** are practically the same, they are just stimuli alone. You can set the duration and activate, for the given duration, light, air puff, auxiliary out (Out 1), white noise (with dB amplitude) and sound (with dB amplitude and frequency): you can set one or more of these types of stimuli.

PREPULSE-PULSE is a combination where a sequence of two stimuli is fired, a pre-pulse and a pulse. IPI duration is the interval that lasts between the pre-pulse and the pulse and a random value between the two limits is used. Just put the same value for IPI Min and Max to set a constant interval.

Protocol Page

Please note that in the PREPULSES (both in the PREPULSE ALONE and PREPULSE-PULSE stimuli) you can also use the GPIAS paradigm (Gap Prepulse Inhibition of the Acoustic Startle). It means that the pre-pulse, instead of being a White Noise fired at a given amplitude, is an interruption (silence) of the background (environmental) white noise for the duration set. The GPIAS, if used, needs that the environmental white noise is activated.

7.3.7. Randomized stimuli
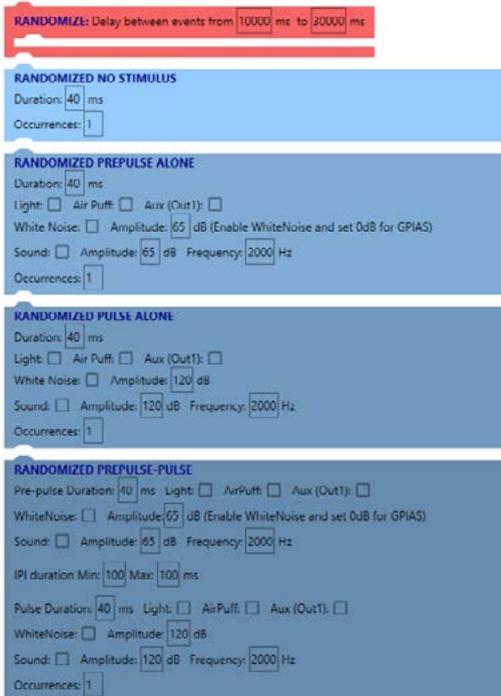
RANDOMIZE: in this block you can put only the following "RANDOMIZED" stimuli and for each of them you must define the quantity of the stimulus to be randomly fired in the block. The delay between the stimuli is randomly chosen between the given limits in the RANDOMIZE block (in red).

If you need a protocol where the same stimuli set (for example 8 different stimuli) must be performed in a pseudo random sequence for example 10 times, you can create a randomize block with the set of 8 different stimuli with a quantity of 1 each and then put this block inside a REPEAT 10 times block (See Paragraph "7.5. Real-world example" a pagina 31).

Details for the stimuli blocks are the same ones described in the previous section "Stimuli". The only difference is that the randomized stimuli blocks have the "Occurrences" field, to define the quantity of the stimulus to be randomly fired in the block.

Warning: Light stimulus is available for mouse platform only.

7.4. Creating the protocol

The protocol script is shown in the main area of the screen. The first block is the purple block called "START".

The next blocks must be attached to this block. To attack a block, you need to drag the desired block and drop it under the previous block, when a green line appears:

Click the left side of the desired block and keep the left mouse button pressed

Keeping the left button pressed, drag the block.

As you can see, while dragging the block, on the left side a waste-basket icon appears, and you can release there the block to delete it.
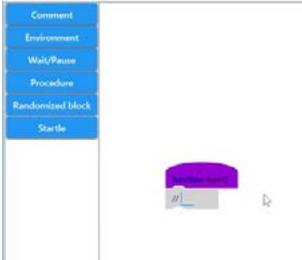
Normally, drag it...

Drag it....

Until you reach the target position, when a green line appears under the preceding block.

While the green line is lit, release the mouse button and the block is automatically connected to the preceding block.

Now you can start to type in or edit the block properties...

In this example it is a comment block, so you can type in the comment

All set.

You can proceed to add the next blocks until you complete your protocol.

7.5. Real-world example

Starting block

Comment: set environment

Start white noise at 70dB

Start Fan

Wait 10 seconds

Start Recording

Basal phase

Repeat 5 times a pulse alone stimulus at 120dB of white noise

With a delay of 5s between them

Start the test

Repeat 10 times

a randomized block of 8 different stimuli with a random delay between 10 to 30s.

In this example we set:
- Prepulse-Pulse WN 73dB and Pulse WN 120dB
- Prepulse-Pulse WN 76dB and Pulse WN 120dB
- Prepulse-Pulse WN 82dB and Pulse WN 120dB
- Pulse Alone WN 120dB
- Prepulse Alone WN 73dB
- Prepulse Alone WN 76dB
- Prepulse Alone WN 82dB
- No Stimulus

One occurrence each. These 8 stimuli will be fired in a random sequence, so every one loop of the 10 set in the repeat block will produce different sequences of the eight stimuli.

Last phase

Repeat 5 times

A Pulse alone stimulus of 120dB White noise

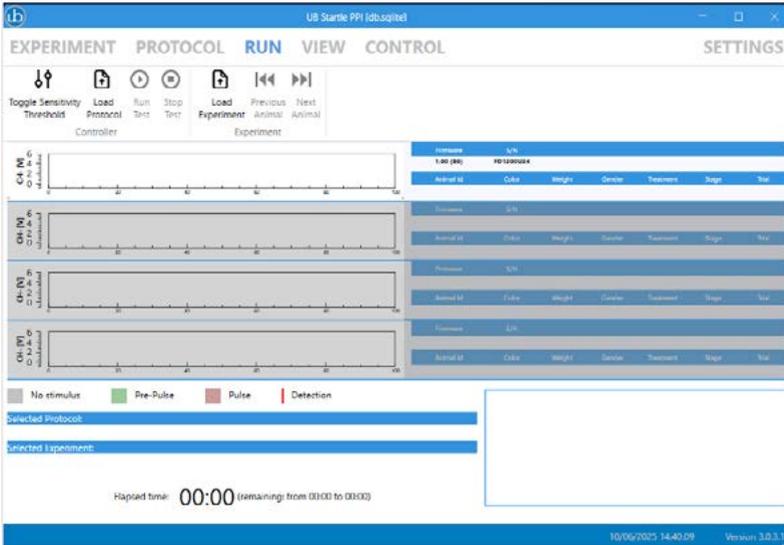with a random delay between them from 10 to 20s

Stop the recording

Stop the environment outputs
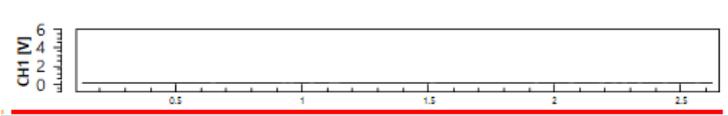
End the protocol

Protocol Page

8. Run Page

This page is used for executing the experiment in the up-to-four connected cubicles.

To the right of each connected cage, the firmware version and the serial number of the cubicle interface is shown.

If a red line is shown under the plot-chart area,

it means that the PPI platform cage is not connected to the black box (the RJ50 cable on the side of the platform is either not connected or not fully into the socket). **After plugging the platform, a restart of the Black-box on the back side of the cubicle is needed (power-cycle)**.

8.1. Toggle Sensitivity Threshold
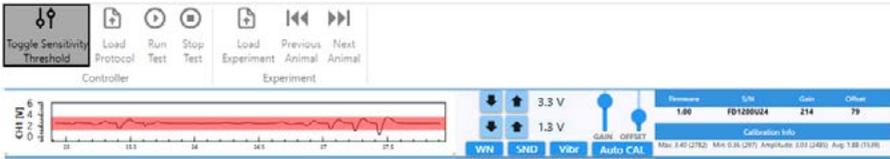
When activating the sensitivity threshold, you can set the upper and lower limits of the "noise" animal movement area. Keep the red line as narrow as possible. Movements whose peaks fall within the red area are not marked as a detection.

While the sensitivity toggle is activated, you can fire a 120dB white-noise pulse (Fire WN button) or a 3KHz sound pulse (Fire SND button), in order to check that the sound air movement doesn't affect the platform or to check the animal startle amplitude.
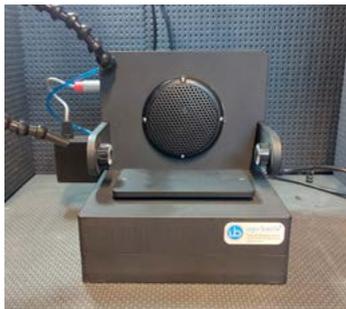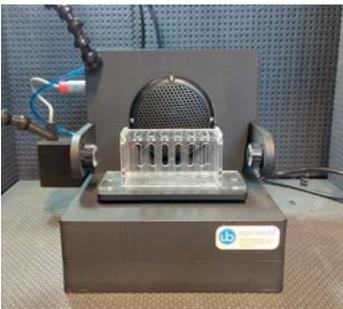
8.1.1. Auto-Calibration

Platform calibration is needed to ensure the correct platform sensitivity for the animal your are going to test; You need to perform the calibration every time you change restrainer model. Calibration also ensure the measurement homogeneity across cubicle/platform
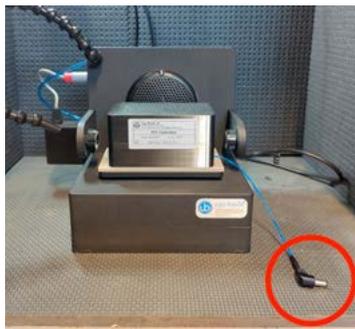
To perform the auto-calibration of the platform, You need to:

1) connect the calibrator:

To connect the calibrator you first need to remove the restrainer from the platform

Then place the calibrator on the platform and connect the calibrator cable to the right side of the platform connector.

2) Into the tab **RUN**, press the button **Toggle Sensitivity Threshold**

Then Press the **Auto CAL** button at the right of the platform diagram you want to calibrate.

Choose mouse or rat and the restrainer model you are going to use for the test.

Then press START.

An automatic procedure takes place, initially calibrating the offset (midpoint) and then tuning the sensitivity thanks to the calibrator vibration.

When the auto calibration has been completed, a "Tuning Completed" message appears:
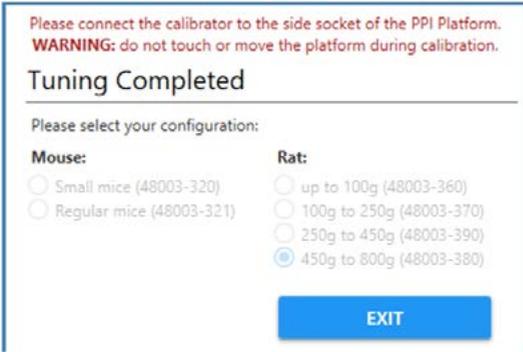
Press EXIT and repeat for all the other platforms.

Note that the calibration gain and offset values are shown on the right side and can be also futherly adjusted manually using the two sliders:
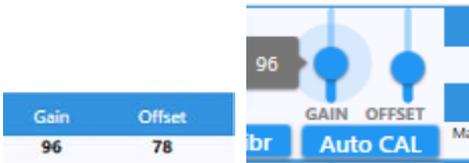
Please note that if you run a multiple cubicle system the manual change of the Gain and Offset on one cubicle will differ from the other cubicle. If You manually change this value you need to manually change them on all cubicle to ensure the same cubicle sensitivity.

After the calibration has been completed, you can remove the calibrator unit and press the toggle sensitivity button to return to normal mode.

8.2. Running a test

Before running a protocol, you must select it. Click "Load Protocol" and select the one you want to run.

Moreover, you can select the experiment, that is the list of animals to be tested, clicking "Load experiment".

Animals are distributed to the active cages sequentially, following the sequence chosen in the EXPERIMENT page. You can change the animals pressing the previous-next buttons. The chosen animal is shown in the right area.

When you are ready, press the Run Test button to start it. A timer indicates the elapsed time and the minimum/maximum remaining time (depending on the random delays set in the protocol).

Every time you run a test, a new session is created and named "PPI_" followed by date and time.

In each connected cage's row, the result of each stimulus is drawn:
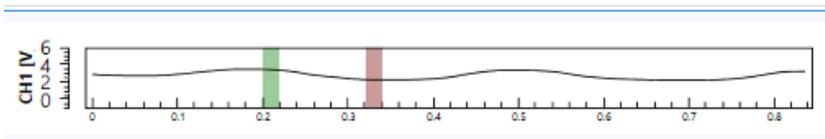
The position of the pulses is highlighted using different colours for prepulse, pulse and no stimulus. This example shows a prepulse-pulse stimulus. As you can see in the example there is a 200ms (0.2s) pre recording time, the stimulus made of a 20ms prepulse, a 100ms inter stimuli delay and a 20ms pulse, then a 500ms capture window is recorded. The total recorded window is 840ms.

In the bottom right block the log of the executed operations is shown:

The leading "|" (pipe) character is used to make evident the nested loops. Each "kind" of execution has its own color.

9. View Page

This page is used to analyze the result data of the experiments.

9.1. Experiment sessions

On the left side, in the "experiment sessions" column, you can select the experiments to be included in the data grid. You can select a single session by clicking its name, or more session using Ctrl-Click or Shift-Click to add a single session or a group of them.

You can also double click a session name if you want to rename it:

The maximum length for the session name is 31 characters as it is a limitation for the Excel file export.

9.2. Data grid area

Enabled	#	Cubicle	Timer	StepName	Stimulus	Session	Protocol	
☑	5	1	423.691	Phase A	Pulse Alone	PPI_20240704_1425...	BIO Protocol.ppi	t
☑	6	1	454.541	Phase B	Prepulse-Pulse	PPI_20240704_1425...	BIO Protocol.ppi	t
☑	7	1	477.861	Phase B	Prepulse-Pulse	PPI_20240704_1425...	BIO Protocol.ppi	t
☑	8	1	507.206	Phase B	No Stimulus	PPI 20240704 1425...	BIO Protocol.ppi	t

In the central area, the data grid contains the list of all the fired stimuli of the selected sessions.

The background of the lines is brownish when there has been a detection.

The columns have a drop-down that allows you to filter the data by one or more columns.

9.3. Plot chart area

When you select a stimulus line in the data grid, the relative curve is shown under the data grid:

Statistics Experiment 'PPI20240704_142559-.5vpp-1.7g' Info

All cubicles totals	AVG Resp.%	Std.Dev.	Std.Err.	Q.ty	Det.
Pulse Alone	54.9	23.71	5.44	20	19
Prepulse-Pulse	37.1	13.11	2.68	30	24
PrePulse Alone	0	0	0	30	0
No Stimulus	0	0	0	10	0
PERCENTAGE OF PPI	32.4				

Enabled	#	Cubicle	Timer	StepName	Stimulus	Session	Protocol	
☑	5	1	423.691	Phase A	Pulse Alone	PPI_20240704_1425...	BIO Protocol.ppi	t
☑	6	1	454.541	Phase B	Prepulse-Pulse	PPI_20240704_1425...	BIO Protocol.ppi	t
☑	7	1	477.861	Phase B	Prepulse-Pulse	PPI 20240704 1425	BIO Protocol.ppi	t

Upper Threshold 2.30 Lower Threshold 1.90 Det. Timeout 0.10

Amplitude mV: 2.314 Duration ms: 27 Latency s: 0.158

Here you can see the recorded period where:
- A green block indicates a pre-pulse
- A red block indicates a pulse
- A grey block indicates a no-stimulus
- A grayed area indicates the detection area

The blue horizontal line (divider) allows to set the vertical size for the data grid and the chart. Just click the arrows and drag the line vertically:

The chart appears only when a stimulus line is selected in the data grid.
If you can't see the data grid or the chart, ensure that the divider is not all the way up or down.

9.4. Stimuli exclusion

In certain circumstances you can decide that a stimulus is wrongly detected, as the reaction was not a real startle but due to other reasons.

In the central grid, the first column headed "Enabled" allows you to toggle the inclusion of a stimulus from the calculations. To exclude (or re-include) a stimulus, just double-click its "Enabled" cell. The entire line is then grayed out to make clear the fact that it is excluded from the calculations.

Enabled	#	Cubicle ▾	Timer	StepName ▾	Stimulus ▾	Session
☑	1	1	60.341		Pulse Alone	PPI_2023062(
☐	1	2	60.341		Pulse Alone	PPI_2023062(
☑	2	1	121.191		Pulse Alone	PPI_2023062(
☑	2	2	121.191		Pulse Alone	PPI_2023062(
☑	3	1	182.051		Pulse Alone	PPI_2023062(
☑	3	2	182.051		Pulse Alone	PPI_2023062(

9.5. Threshold adjustment

In the previous mentioned curve, you can change the thresholds if they were not good for a specific stimulus. Increasing it determines a higher sensitivity but also the risk of false positives. The opposite is true if we decrease it.
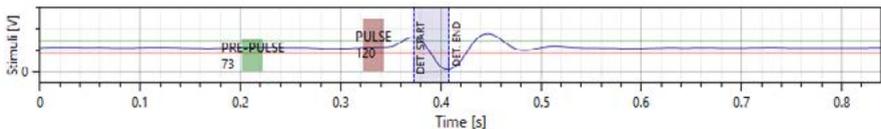
Upper Threshold 2.8 ↕ Lower Threshold 1.7 ↕ Det. Timeout 0.20 ↕ **Save only this** **Save All same Session+Cubicle**

Amplitude mV: 3.039 Duration ms: 35 Latency s: 0.173

If you keep the Ctrl (control) key pressed while adjusting the thresholds you can fine-tune the value (instead of incrementing / decrementing by 0.1, you'll do by 0.01).

If you change them, a "Save only this" and a "Save All same Session+Cubicle" buttons appear.

Clicking "Save only this", the new thresholds and detection time-out are saved to the database only for the selected stimulus.

Clicking "Save All same Session+Cubicle" the new thresholds and detection time-out are applied to all the stimuli for the current selected record's session and cubicle.

9.6. Exporting data

If you want to export to excel only selected sessions, select them in the left area and press "Export selected" button. Otherwise, all the sessions are saved.

For each session both a csv and a xlsx files are created. The xlsx file contains additional sheets: the second contains the raw data and the last contains the startle data.

Clicking the "Select fields to Export" button in the top menu, you can choose which fields you want to be included in the CSV/XLSX file.

You can click "Select All" or "Deselect All". Please be aware that some fields are mandatory and cannot be excluded as they are needed to recognize uniquely a stimulus or contain the startle measures.

9.6.1. Raw data (XLSX only)

It's a value between 0 and 4095, where 2048 is the mid-line (2.5V) and 4095 is 5V. A value every 1ms is given.

9.6.2. Startle data (XLSX only)

A value every 1ms is given. Only values different than 0 are significant:

- 1 means pre-pulse
- 4 means pulse
- 5 means no-stimulus
- If the value is greater than 15, it is the detection start point.

9.7. Statistical data

Selecting the "Statistics" tab on top of the data grid, a brief summary is shown:
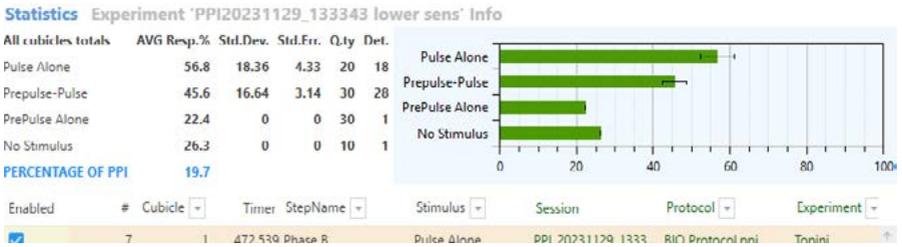
Here, the average, standard error, and standard deviation of the stimuli responses are shown. Only the stimuli with a detection are considered. The percentage of PPI at the bottom is the relationship between the pulses alone and the pulses preceded by a Pre-Pulse. "Pre-Pulse alone" and "No Stimulus" stimuli are not taken in account in this calculation.

The statistics include all the currently selected sessions.

10. Control Page

This page is used to test the various environment and stimuli in the cubicle. We suggest a quick check to this page before the tests.
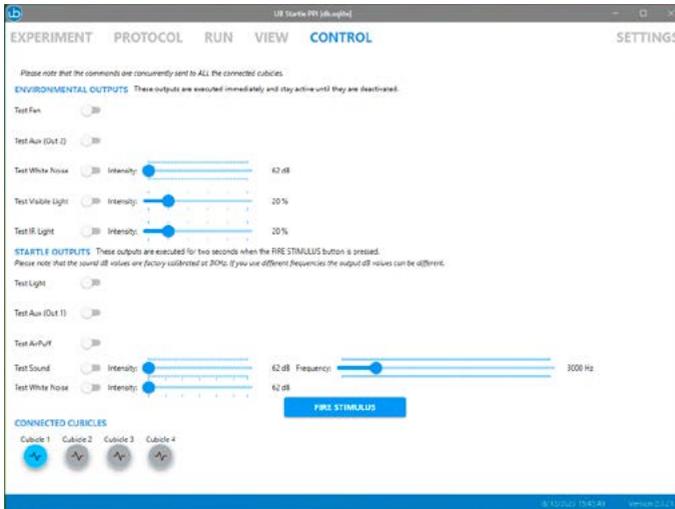

The environment outputs are executed immediately.

The Startle outputs are fired for two second when you press the "FIRE STIMULUS" button.

This is just for testing purposes.

The two Aux outputs can be used to connect external devices. Out 1 is tied to the stimulus and the Out 2 is environmental.

To connect the auxiliary outputs to external devices/dataloggers, the optional "I/O Box for TTL signals" must be used (SKU 46000-170). This I/O box provides a total of four TTL inputs and four TTL outputs. The Startle PPI system uses only the first two outputs, the other TTL outputs and the four inputs can be used with Any-maze if the cubicle is also used for Fear Conditioning.

If you click the cubicle round button in the CONNECTED CUBICLES area, the Startle PPI Cage light flashes twice and a low-volume sound is fired. This can help you to identify the cages as the numbering of the cubicles may depend on the order in which they are connected to the USB ports. How to change the devices enumeration is described later on in this document.
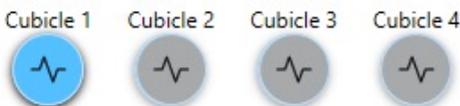
11. Settings Page
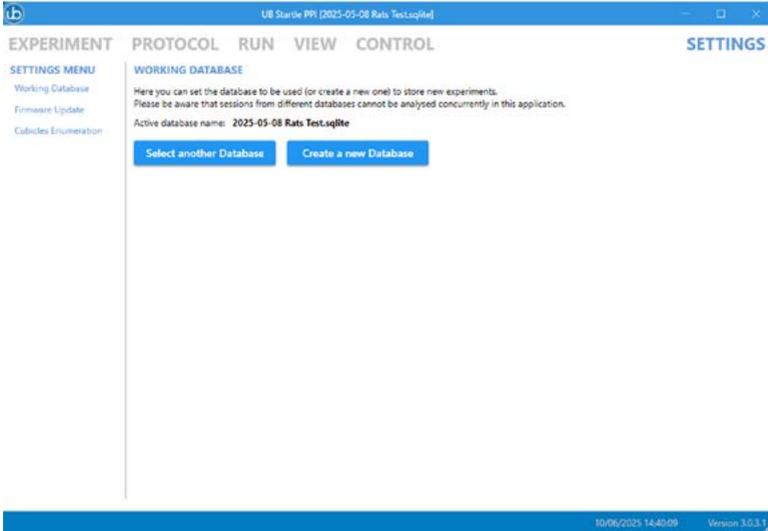
11.1.Working Database

In this page you can select the database to be used to store the recorded data.

Be careful using this feature as you cannot view or compare data from different databases.

Here you can select a new database (the default one is called "db.sqlite"). You can choose to switch to a previously used database or to create a new database.

If you want to create a new database, its name must follow the file naming rules, so the following characters are not allowed: < > / \ | * ? : ".

Moreover, the database MUST reside in the default folder (in the "Documents\ StartlePPI\DB folder"). If you try to open a database that resides outside this folder, a warning appears. Please copy the needed database in that folder and then you can open it.
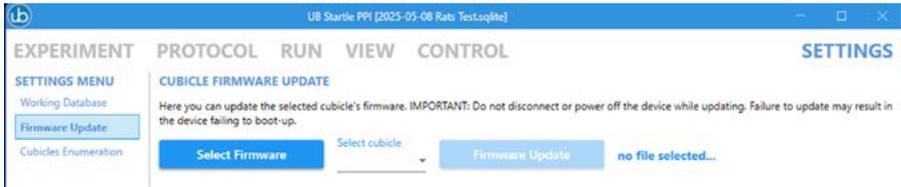
Please note that the currently selected database name is always visible in the application title bar (enclosed in squared brackets):

11.2.Firmware Update

This functionality allows you to update the firmware of the selected cubicle.

The update file must be placed in the Documents\StartlePPI\Updates folder and its name has a ".srec" file extension.

Here you should select the firmware file, select the cubicle you want to update and then click "Firmware Update" to start the operation. It can take up to 5 minutes to complete.

In case any error happens during the update, the old version is maintained.

Please give a look to the update log for errors or warnings. If you encounter any problem, please contact the Ugo Basile service team describing the error message you received.

Once the update completes, please switch to the "RUN" page and verify that the device reports the new firmware version:
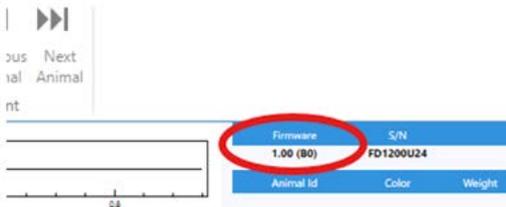
If you have multiple cages, please proceed with all of them. All the cages must have the same firmware version when used together.

11.3.Cubicle Enumeration

Use the Settings page to configure the cubicle sequence:

Select "Cubicles Enumeration" from the menu on the left.

You can rearrange the cubicles by selecting one and moving it up or down using the corresponding buttons.

To identify a specific cubicle, simply double-click its serial number to play a sound in that cubicle.

Once you're satisfied with the new arrangement, click SAVE to confirm and apply the changes.

12. Maintenance

While any service of the instrument ought to be carried out by Ugo Basile personnel or by qualified personnel authorized by UGO BASILE organization, this manual section describes normal maintenance procedures which can be carried out at your facility.

UNPLUG THE MAIN CORD BEFORE CARRYING OUT ANY MAINTENANCE JOB

12.1.Cleaning/disinfection

The Startle PPI does not require any maintenance apart from normal cleaning.

Do not use organic solutions. Cotton wool and water can be used for cleaning purposes. For disinfection, use a non-alcoholic disinfectant, or H2O2.

12.2.Long Inactivity

The instrument does not require any particular maintenance after long inactivity, except cleaning.

12.3.Customer Support

For any further information you may desire concerning the use and/or maintenance of the Startle PPI, please do not hesitate to contact our service department (or our local distributor) either directly or via our support web form.

Customer Support Contacts

Phone: +39 0332 744574

E-mail: service@ugobasile.com

Web form: https://ugobasile.com/support/support-request

Before sending the instrument to our factory for repair, please contact our logistics department to obtain a return authorization number (RMA) and shipping/packing instructions. We may not be held responsible for damages during transport due to poor packing; whenever possible, please use the original packing.

13. Specification

Operation	
Sound Range	From 65 dB to 120 dB
Pulse/pre pulse sound range	From 65 dB to 120 dB
Sound Frequency Range	From 100 Hz to 18 KHz
IR Light	0-100% (Environment)
Visible Light	0-100% (20K Lux) S/R Platform)
Data Export	.csv, .xlsx format
General	
Power Requirements	Universal input 100-240 VAC, 50-60Hz, 60W
Background Noise (Ventilation)	from 0.1 to 3mA, in 0.1mA steps
Operating environment	10°C to 40°C; 5% to 95% RH (non-condensing)
Physical	
Cubicle dimension, ouside	52(d)x62(w)x58(h)cm
Cubicle dimension, inside	39(d)x47(w)x49(h)cm
Shipping Weight	Mouse Single-Cage Set-Up: 40Kg
Packing Dimensions	77x65x83 cm (wooden crate) for a Mouse Single-Cage Set-up
Warranty	
Warranty	Startle PPI is covered by a 12-month warranty + 12 after product registration

14. Bibliography

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Marsh, R.R., et al., (1975) "The Role of Small Changes in the Acoustic Environment in Modifying the Startle Reflex" J Exp Psychol Anim Behav Process, 1(3)

15. Related products

Since more than 50 years Ugo Basile designs and manufactures classic and innovative devices, essential in animal behavioral studies.

Whether your research involves the study of memory, learning, anxiety, depression, fear, stress, social interaction, addiction, or more complex behavioural protocols, we have the answer: our line of conditioning/avoidance cages, mazes, videotracking, etc. keeps growing thanks to always new devices and methods.

46101- Fear Conditioning System

46000-101 - Electric Shock Meter

49603 - Operant Fear Conditioning

49500/49503 - Operon (for attentional set-shifting)

42552/42553 - Conditioned Place Preference Cages for Rats or Mice

47502/47503 Learned Helplessness

What's in the box

40532/40533 - Active Avoidance set-up for Mice and Rats (Shuttle-Box)

40570 - Passive Avoidance - Step Down for Mice (vibrating platform)

40550 - Passive Avoidance - Step through - for Mice and Rats

45100/40150 - Lickometer (Vogel Test) set-up for Drinking-Conflict experiments

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Ugo Basile SRL Società Unipersonale
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Tel. +39 0332 744574
sales@ugobasile.com
service@ugobasile.com