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# Assessment of prepulse inhibition (PPI) of the acoustic startle reflex in rodents

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**We use this protocol and it's working**

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**Keywords:** acoustic startle reflex in rodent, prepulse inhibition, assessment of prepulse inhibition, intense acoustic stimuli, acoustic startle reflex, important indicator of sensorimotor gating, deficits in sensorimotor gating, startling stimulus, sensorimotor gating, dopaminergic neurotransmission, timing of the stimulus, schizophrenia, sensory information, involuntary response, misinterpretation of sensory information, ppi in mice, individuals with schizophrenia, stimulus, sensory overload, brain, such as loud noise, weak prepulse, alertness

## Abstract

The acoustic startle reflex is an automatic and involuntary response that occurs in humans and other animals when they are exposed to sudden and intense acoustic stimuli, such as loud noises. This response can be influenced by several factors, including the intensity and timing of the stimulus, the individual's state of alertness, and contextual factors. It can also be modulated by prior exposure to a weak prepulse shortly before the startling stimulus, a phenomenon known as prepulse inhibition (PPI). PPI reflects the brain's ability to filter out irrelevant or non-threatening sensory information, and is considered an important indicator of sensorimotor gating. In individuals with schizophrenia, both the startle response and prepulse inhibition are disrupted, which is believed to result from alterations in glutamatergic and dopaminergic neurotransmission. These deficits in sensorimotor gating may lead to sensory overload and the misinterpretation of sensory information. Here we describe a protocol to measure PPI in mice and rats using a startle chamber from Insight® (SP, Brazil).

## Materials

### Software

| | |
|---|---|
| Monitor de Sobressalto para Ratos e Camundongos | NAME |
| Windows | OS |
| Insight Equipamentos Ltda | DEVELOPER |

### Equipment

| | |
|---|---|
| EP-175 Startle Sobressalto | NAME |
| Insight Equipamentos Ltda | BRAND |
| N/A | SKU |
| https://www.insightltda.com.br/produto/ep-175-startle-sobressalto/ | LINK |

## Troubleshooting

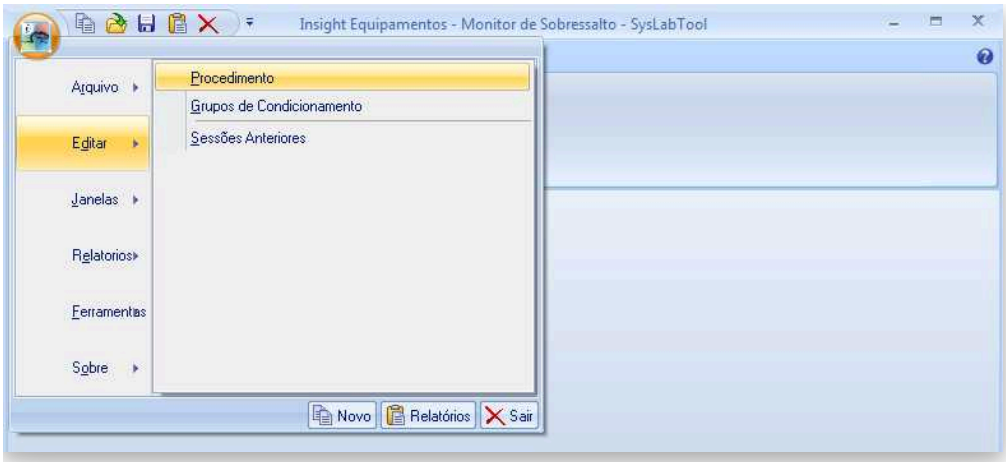
## Before start

This is a step-by-step guide developed at PsychoLab to set up the software used to assess prepulse inhibition (PPI) of the acoustic startle reflex in mice or rats using the equipment acquired from Insight Equipamentos Ltda.

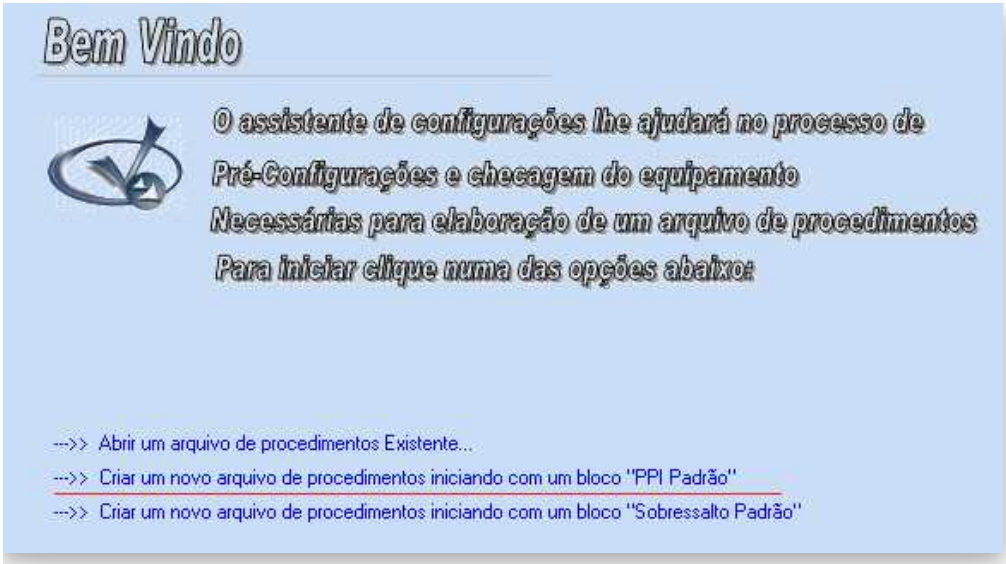
- Make sure that the speakers and ventilation of the apparatus are turned on;
- In our protocol, we did not use any light source inside the box;
- Read the entire protocol before starting.

## Setting up the protocol

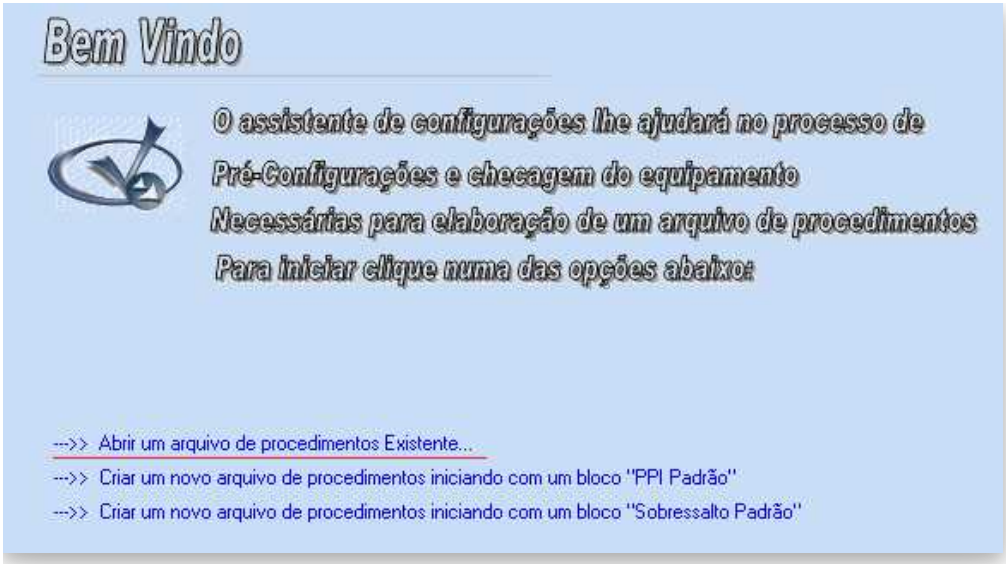
1 **To create a new protocol**, click on the logo in the upper left corner and select **"Edit"** and then **"Procedure"**.




- To create a new prepulse protocol, select "Create a new procedure file starting with a standard PPI block" and name the protocol.




- **If you already have a saved protocol file**, select "Open an existent procedure file" and then select the file *(example.sbs)*.




- Examples of complete procedure files can be found attached below.

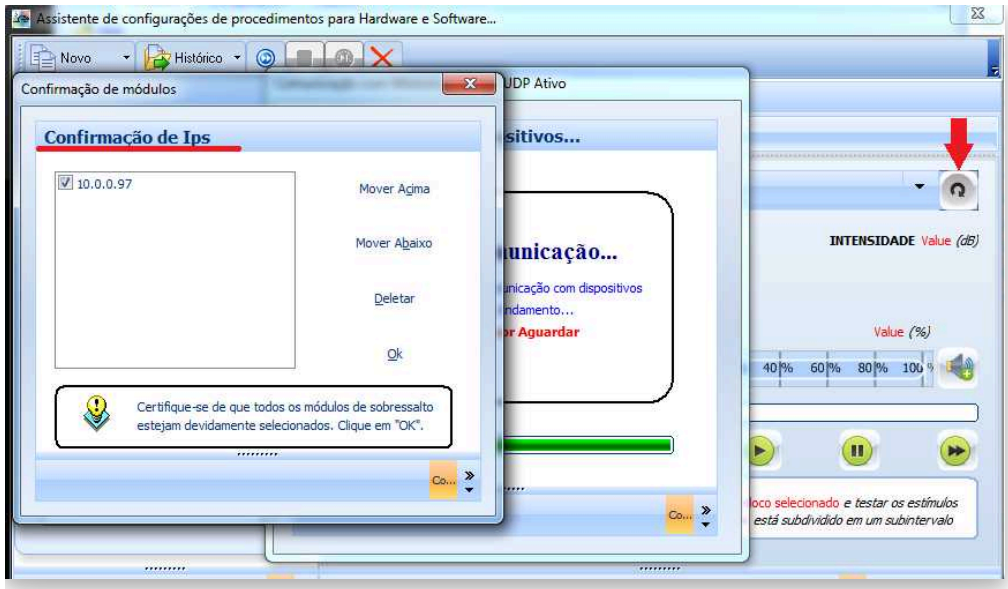
Standard PPI.sbs Habituation.sbs

2 **To calibrate the background noise,** you need to first place the decibel meter inside the equipment, just above the accelerometer.

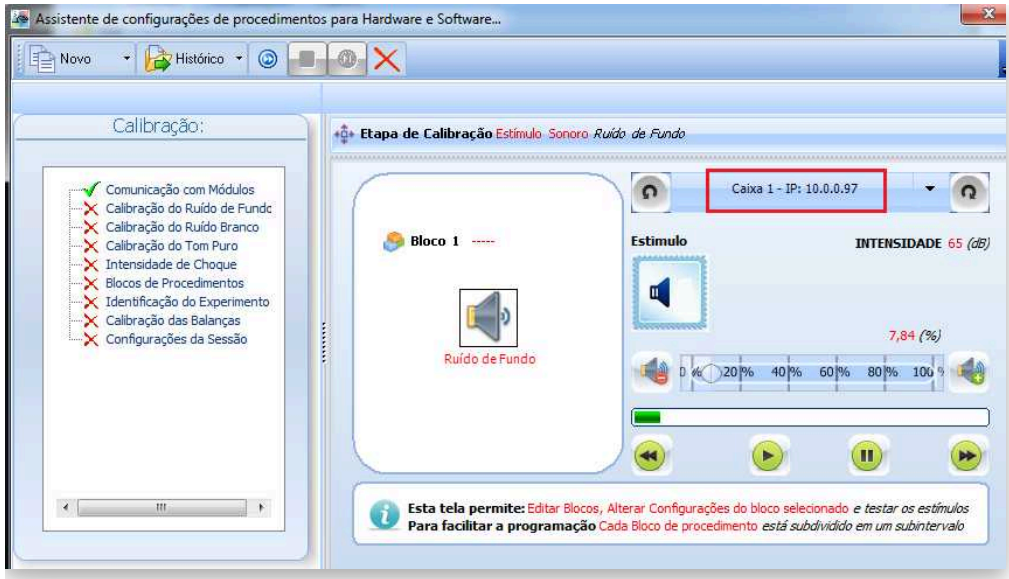

- It is essential to check if the decibel meter's battery is charged or new. A low battery can impact sound detection;
- Position the decibel meter on the accelerometer and adjust the detection range as necessary (range 30-80 dB for background noise; 80-130 dB for other measurements);


- In order to see the readings of the decibel meter, remove the lid of the equipment and close the door.

3 **Select the speaker** of the equipment to calibrate the background noise.




The button to select the speaker is pointed by the red arrow. Speaker identification will be shown in the box "Confirmação de Ips" for selection.



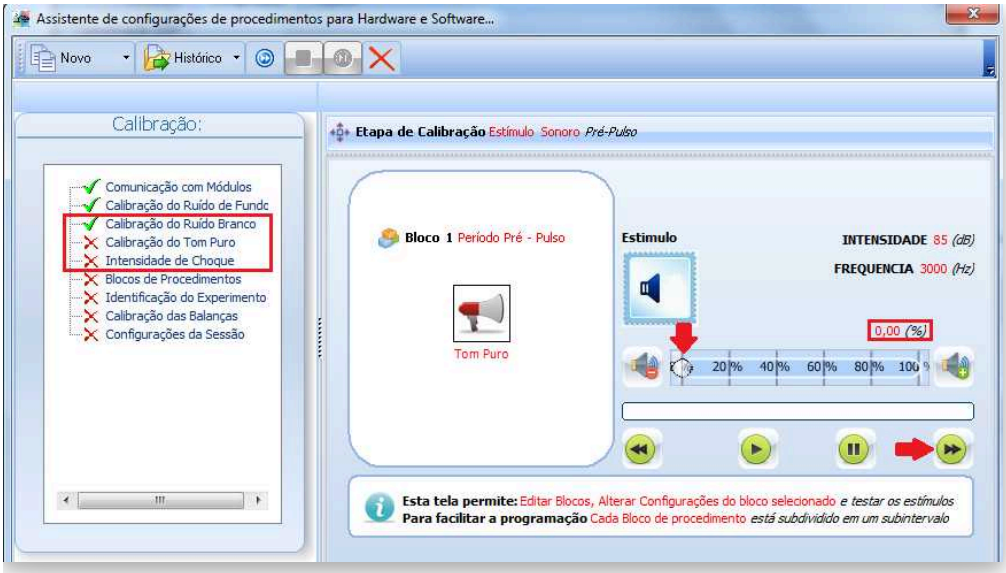

- The number beside "intensity" does not affect the sound range, it is merely decorative;

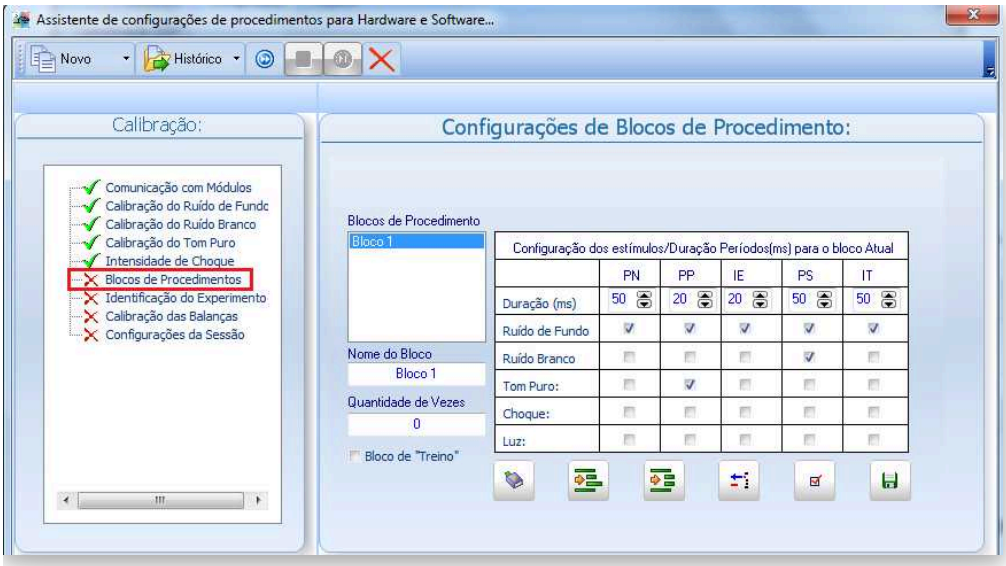

- Adjust the intensity of the sound manually in the percentage bar and press play. Check the decibel meter inside the equipment to see if the desired range is shown. If it is, click on the next button. You can adjust the sound range with the buttons at the beginning and the end of the percentage bar, or move it freely with the mouse by sliding the bar;


- You can adjust the percentage as many times as necessary. Click on the start button to see if the range shown in the decibel meter is right. For other stimuli, just repeat the process outlined in step 2 until you have set everything up go to step #2 ;


- **We advise you to take notes of the percentages** shown in each stimulus. It will facilitate stimuli calibration.

4 **After clicking next,** set the percentage bar to zero in the next three parameters - pulse, prepulse and shock, as we are first calibrating background noise. Click on the next button until you reach the **Block Procedure Settings** screen.






5 In the left side of **Block Procedure Settings** screen, you will see a list entitled **Procedure Blocks** and the **Stimuli Settings** table at the center.


- This software uses the nomenclature blocks as stimuli, so you will see in the procedure block list the first block (stimulus) that we have already configured, the **background noise.**

- Just below this list, you can **name the block** (stimulus) and **set how many times it will be played**. The name set here will be available when you export your data. As we have configured background noise, it could be named "no stim" or "bgd noise", for example.

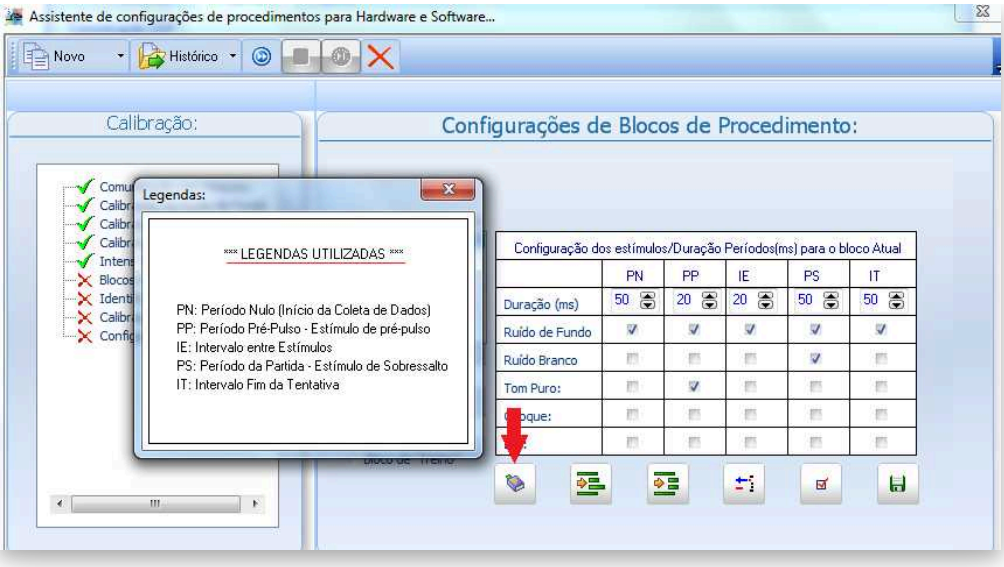

- If the stimulus should be a training stimulus and not enter the analysis, you can go ahead and check the **"training block"** at the end of this column. This is especially relevant for protocols in which animals are acclimatized to stimuli at the beginning and at the end of the test.


6 **To configure stimuli settings,** go to the table and set the duration of each period in the first line according to your protocol. Each stimulus (Block) will have its own configuration regarding background noise, white noise, pure tone, shock and light (lines 2 through 6, respectively).


Duration settings


Presentation settings

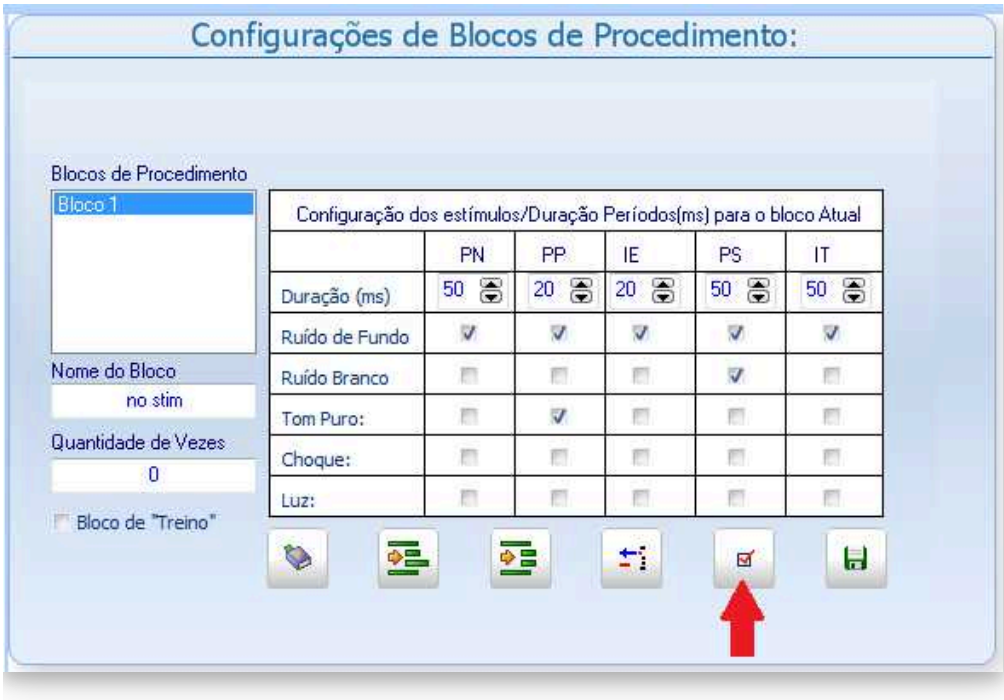
- You can find a more detailed explanation of the columns' abbreviations in the first icon on the left corner, just below the table.



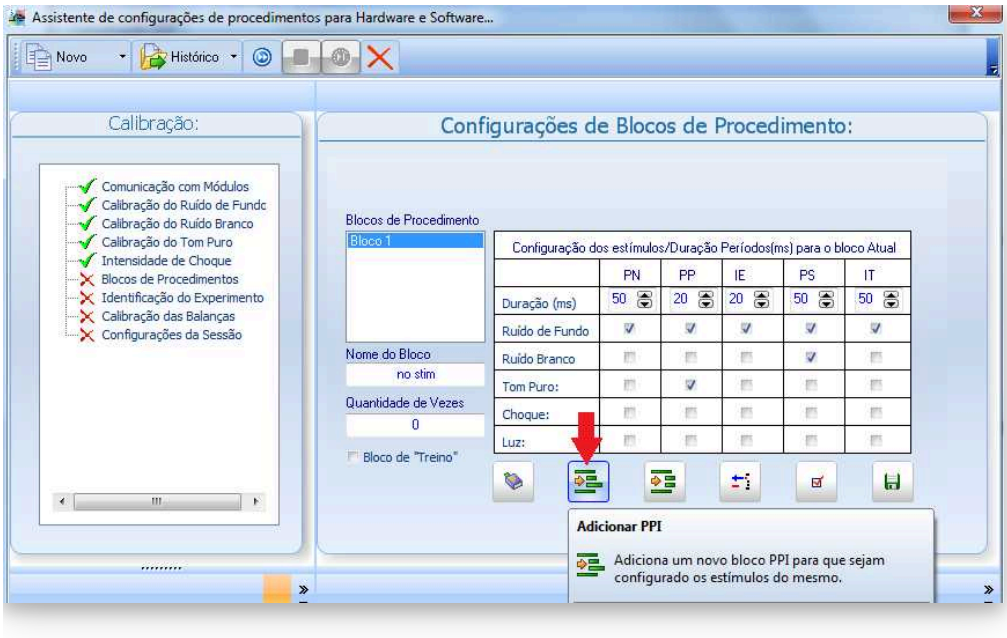

- **Background noise** is typically present in all stimuli, so using it as an example, **all** the boxes in the background noise line should be checked so it is played in all periods of the trial;


- For a **pulse stimulus**, for example, in addition to background noise checked in all periods, white noise should be checked in the **PS column.** For **prepulse** stimulus, pure tone should be checked in the **PP column.** Finally, for a **prepulse + pulse** stimulus, white noise should be checked in the PS column and pure tone in the PP column.


- **Important!** Once you have corrected input the stimulus settings for each block, click on the checked box icon below the table, on the left side of the floppy disc icon. This will guarantee that your settings for that stimulus are applied and saved.

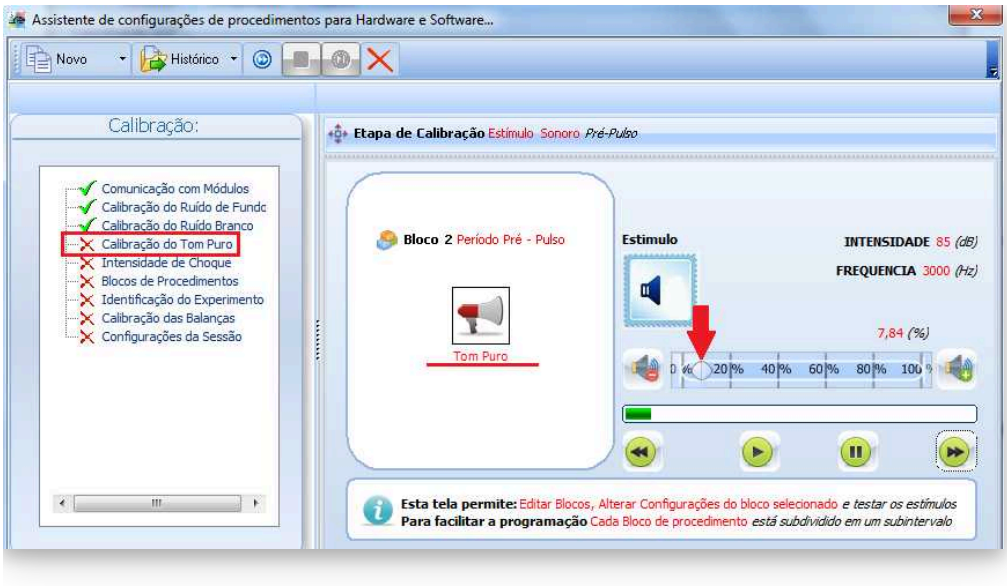


7 **To create a new stimulus** (hereby called "Block"), click on the "add PPI" icon, it is the second icon from left to right below the table, on the right side of the caption icon.

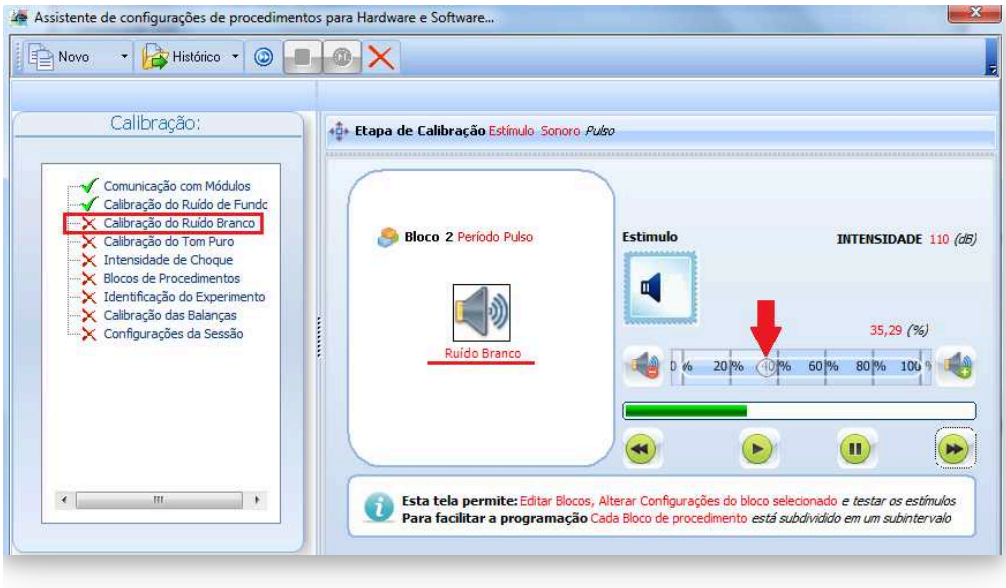



- A new block will be created and you will repeat the steps described in **step 3**, but this time, pulse and prepulse intensity should be set at the desired percentage according to your protocol. For more information on how to set a pulse or prepulse stimulus follow the steps below;

- For a **prepulse stimulus**, in addition to background noise, you will also set **pure tone percentages** in the prepulse period**.** Do not forget to adjust the decibel meter according to the range that you are configuring;  you will set background noise and prepulse period with pure tone;




- For a **pulse stimulus**, you will set background noise and pulse period with white noise;

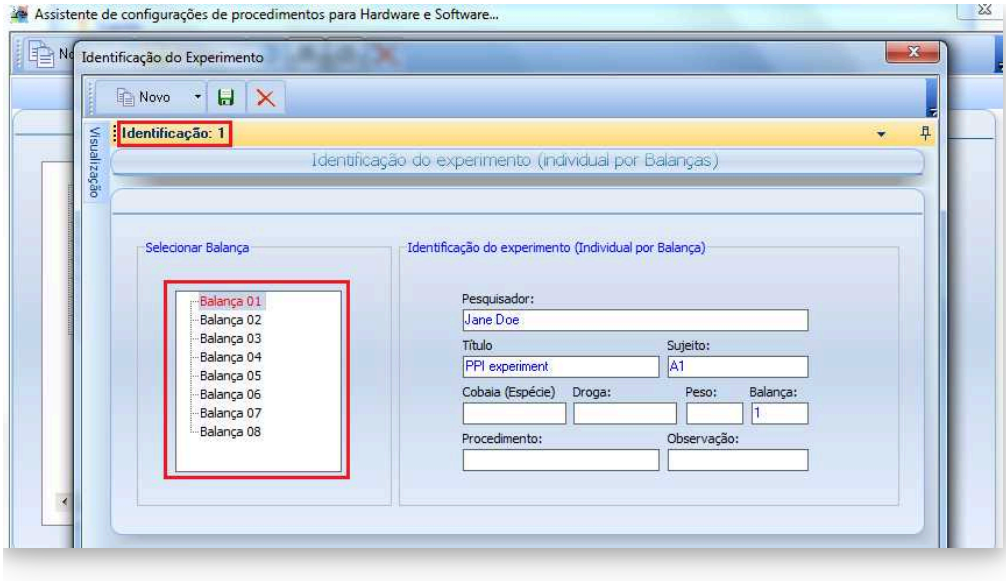



- For a **prepulse + pulse stimulus**, background noise, pulse period and prepulse period should be set;


- Do not forget to take notes of the percentages used - you can find the right percentage for each stimulus just once and then apply it to the rest of the combinations;

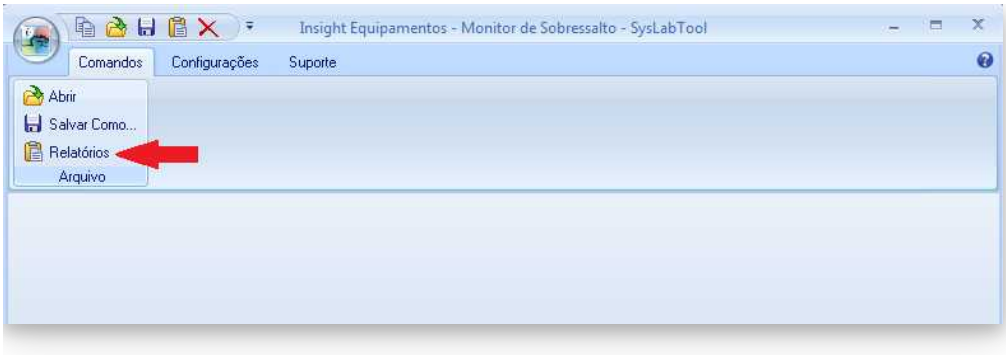

- You should create as many stimuli as needed, so, for example: if your protocol has 15 unique combinations of stimuli, you should create 15 blocks of PPI and calibrate them individually.

8 **Once all stimuli are created,** click on the floppy disk icon on the far right below the table. Experiment identification window will open. The scale used by the equipment is number one. The data entered here will be exported in the results report, available at the end of each test.



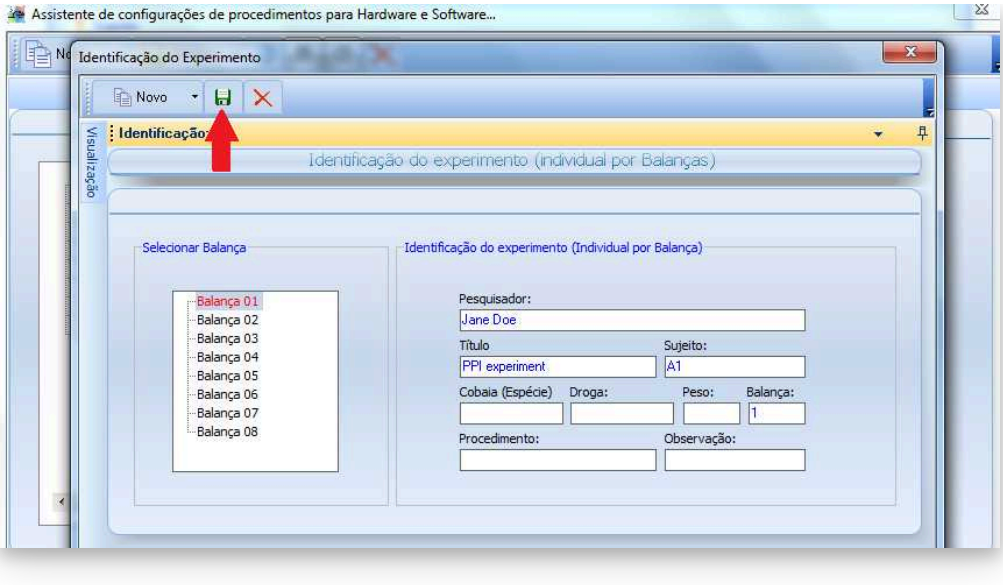

- **Always take note of the identification number**, just above the experiment identification headline. The equipment storages up to 100 subjects' data. If, for example, you are starting your test at ID 93 and will test ten subjects, the equipment will automatically count subsequent subjects in crescent order, but it would replace ID 100 three times since you have 10 subjects and only 7 data spots. In cases when ID spots are insufficient, you should reset ID count.

- **Resetting ID count** will not erase previous data. To do that, click on **"Reports"** on the main screen. The data bank window will pop up.

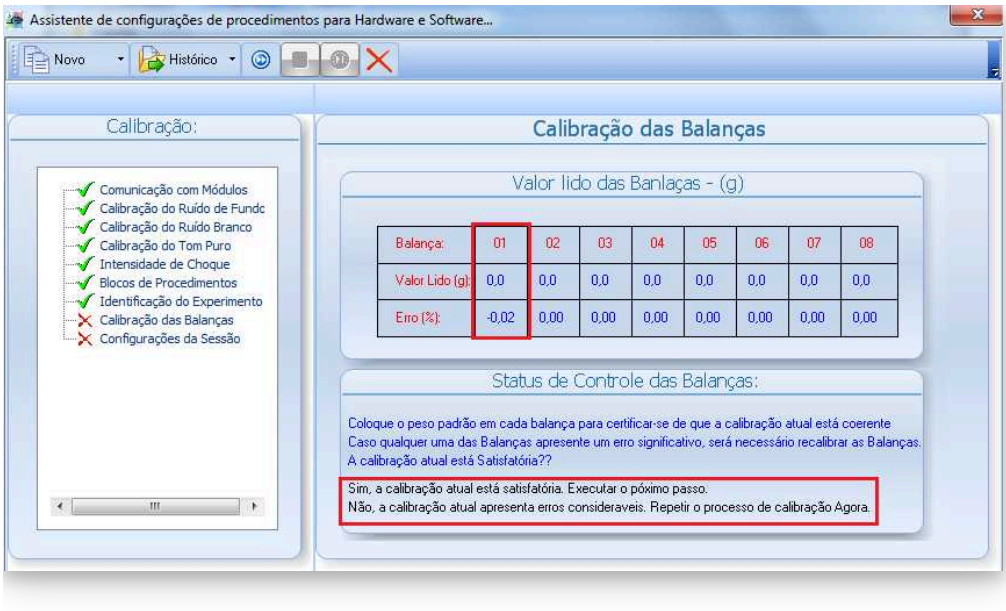


- Be aware that this action will not erase previous data, but can not be undone. If you are sure, click on the penultimate icon on the top bar and the ID count will be reset.

- The data filling here works as a complement to better identify your subject, although we advise you to at least identify yourself (researcher) and the subject, it is not necessary to the test that you fill this part. When finished, click on the floppy disk icon on the left-up corner to save and **exit the window**.

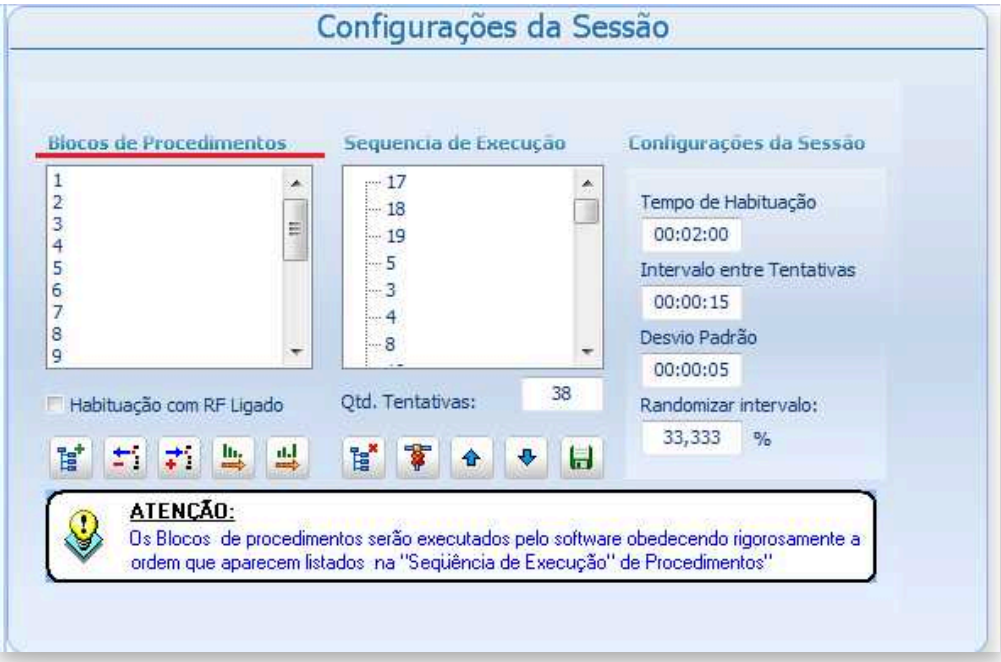



9 **Scale calibration** is an important step to ensure that data collection of startle responses is accurate. First, remove the decibel meter from the scale if it is still inside the chamber. At the beginning of the test, you will click on the option "No, scale calibration presents considerable errors. Repeat scale calibration process now".




- Follow the process shown in the new window. It is recommended to use clean and dry gloves when handling the 50-gram weight. The scale calibration weight can be found within the apparatus;
- After completing the process, check whether the scale accurately displays the weight of 50g and if it resets to zero when the weight is removed;
- You can calibrate the scale at the beginning of the tests and then watch carefully in every session if the measuring is consistently accurate (i.e, resetting to zero without fluctuations). If it is, you do not need to calibrate the scale again;
- After finishing scale calibration, you should click on "Yes, current scale calibration is satisfactory. Execute the next step".

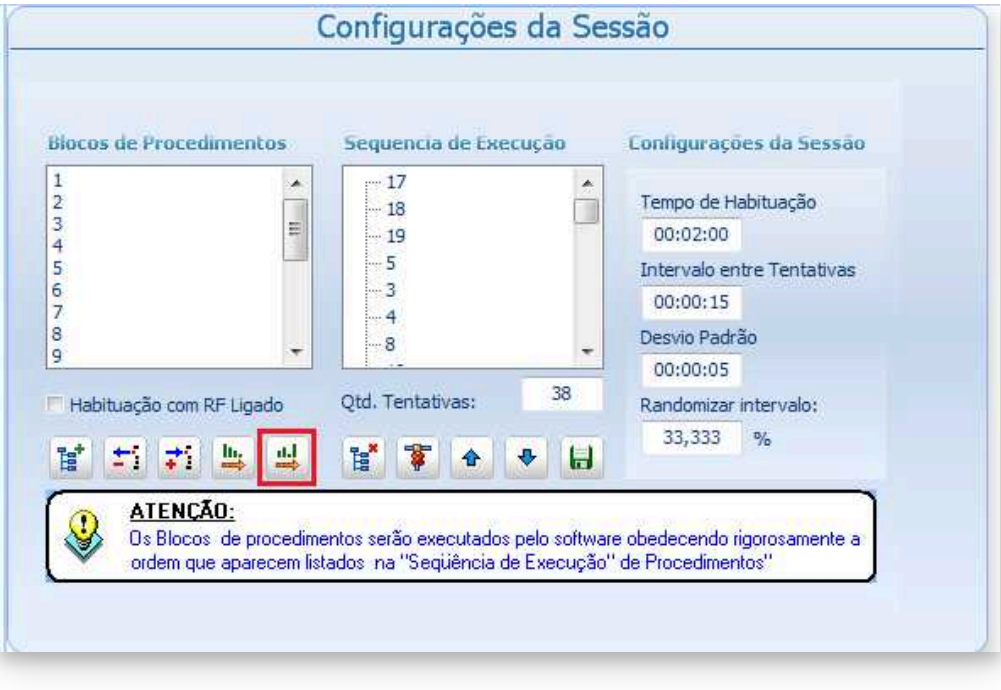
10 **Session configuration** is where you will create stimuli presentation order, which will be exactly the same in all sessions. In the **procedure blocks column**, you will see the previously created blocks (stimuli) just above the "Habituation with background noise on" box.




- If you want the **habituation period to have background noise on** (standard background noise, not configurable) check the box below the first column. If you do not have a habituation period foreseen in your protocol or prefer silence, you should leave the box unchecked;

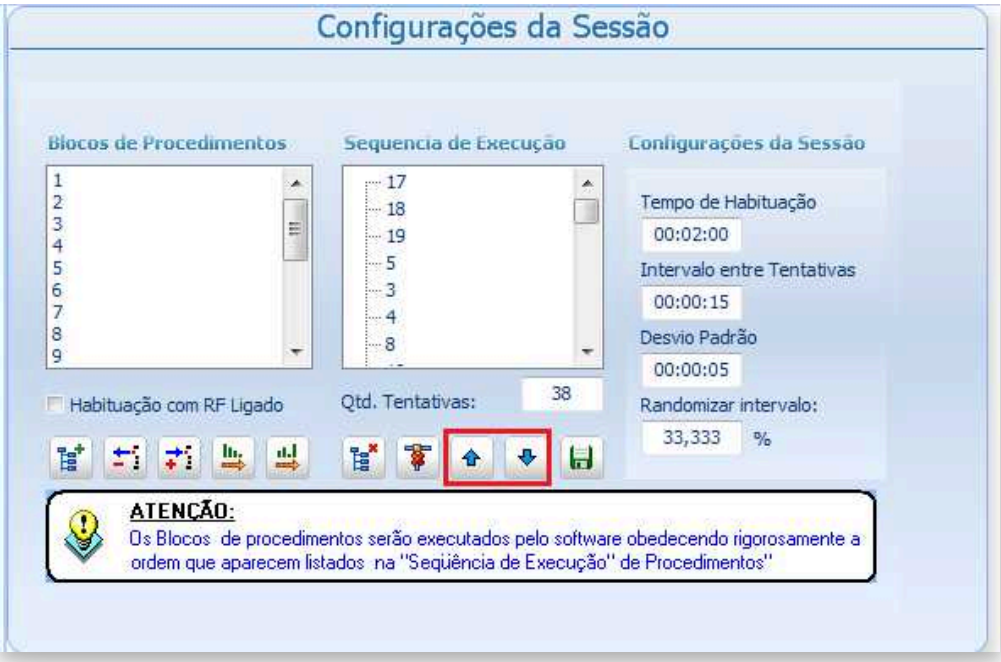
- **To configure the session**, you will transfer the blocks (stimuli) from the procedure blocks column to the **execution session column**. To do that, you can select the block(s) and press "Export selected blocks", which is the fourth icon below the first column. This will transfer blocks **in the same order** as they are seen in the first column. To **transfer a single block** to the second column you can use the third icon, and to **delete a block from the second column,** press the second icon below the first column;


- You can also **transfer blocks randomly**, using "Export blocks randomly", the fifth icon below the first column;

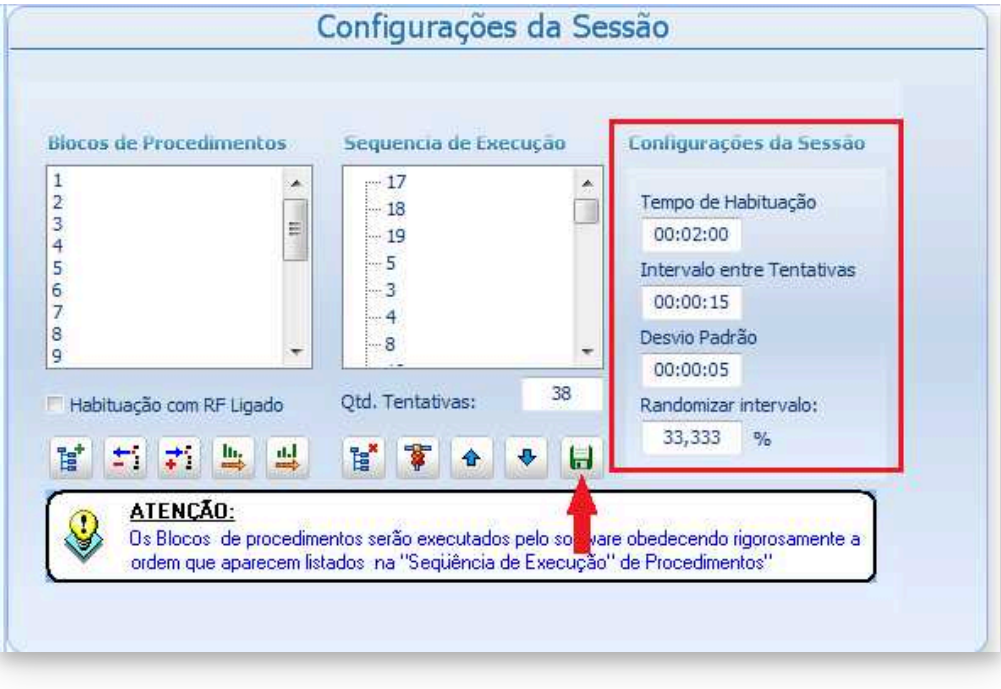


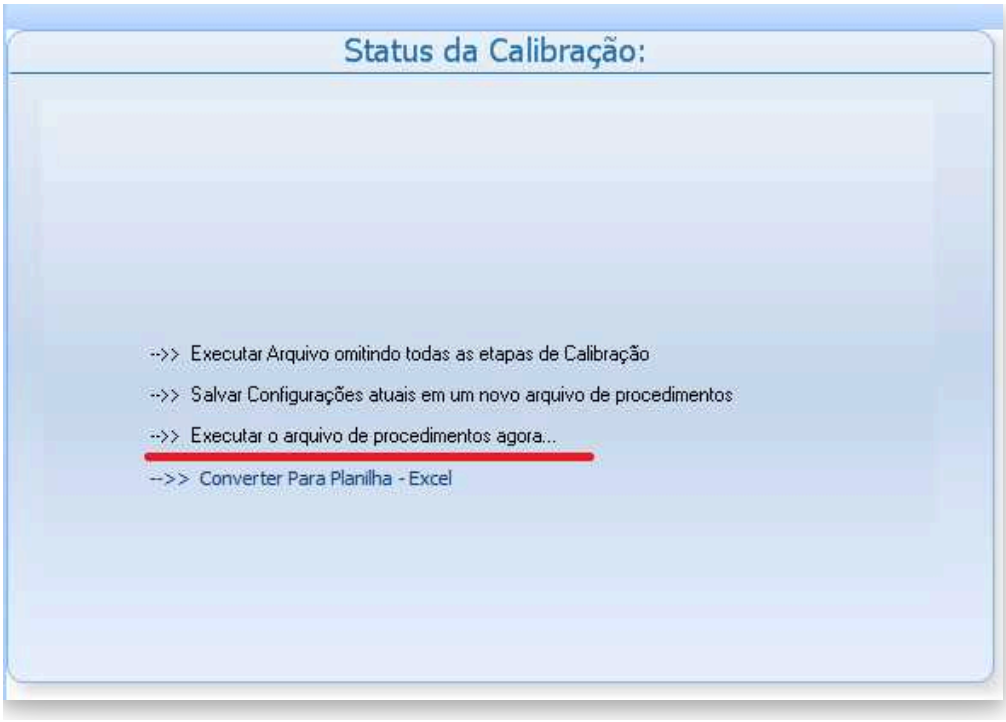

- Please note that blocks will be exported accordingly to the configuration performed in **step 5** go to step #5 , so **if the block was set to be repeated 10 times, it will be exported 10 times** (orderly or randomly, depending on which exporting icon you choose);

- To **move blocks** in the second column, use the arrows below the execution session column, as necessary. To **delete selected blocks** in the second column, use the icon on the left side of the up arrow. To **delete all blocks** in the second column, use the first icon below the column.




- Lastly, to conclude the **session configuration,** are the parameters shown in the far right column - habituation period, time between trials and standard deviation. You should fill in these parameters accordingly to your protocol. To finish the session configuration, click on the floppy disk icon and click on the **"Execute procedure file now"** in the new window that will pop up.



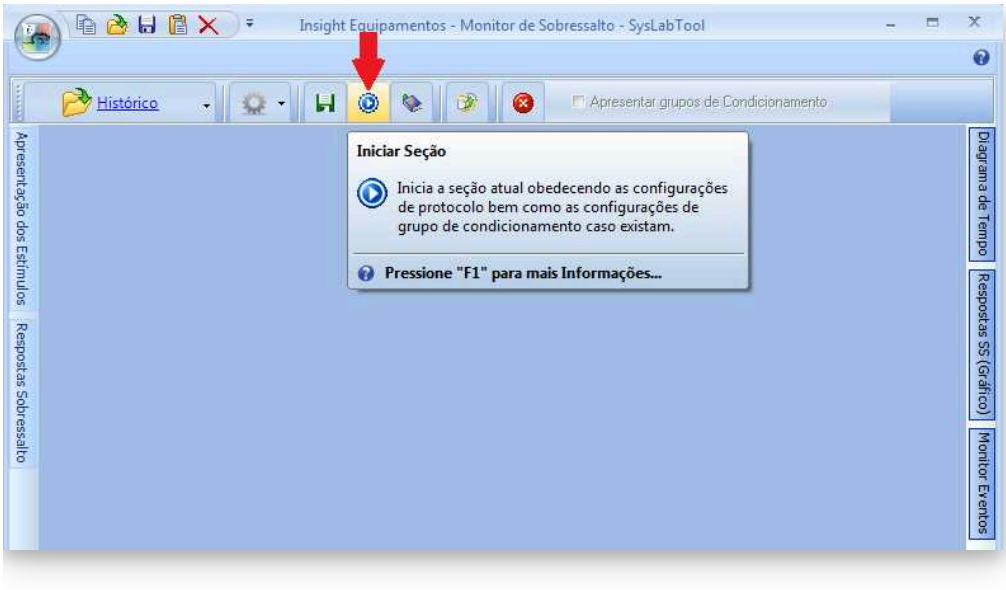



11 **Adjust scale gain** by clicking on the left inferior tab of the main screen. Accordingly to the manufacturer, scale gain should be as described below.

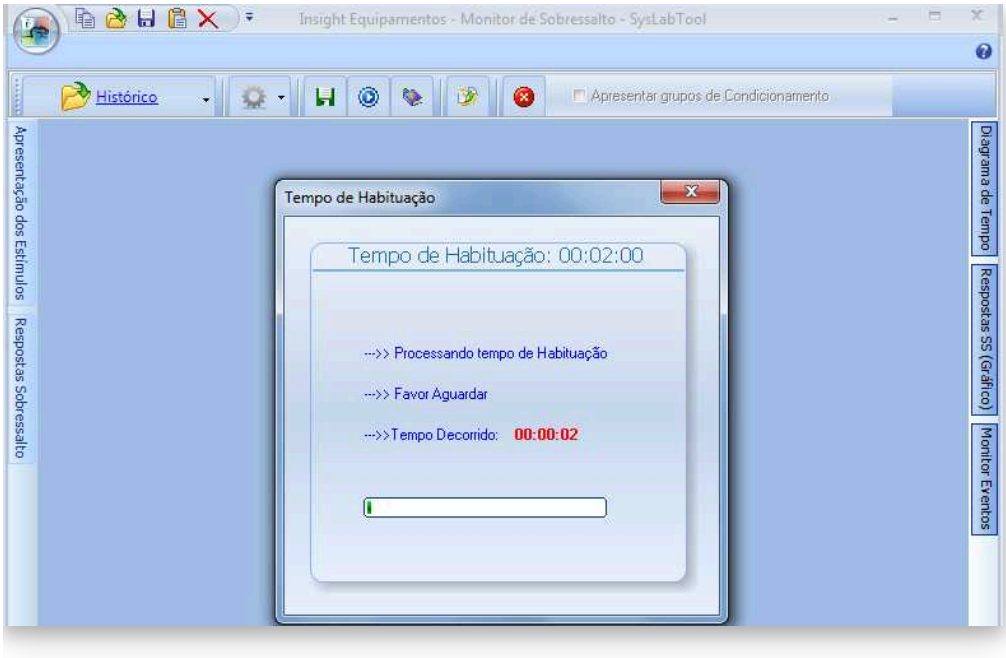

- For mice, scale gain should be set to 1;
- For rats weighing up to 200 g, scale gain should be set to 2;
- For rats over 200 g, scale gain should be set to 3.

## Running the protocol

12 **To start the test,** put the subject in the apparatus and place it just above the scale. **Make sure the lid is on and close the door**. Then, click on the play icon tab, on the upper left side of the screen. A new window will open so you can choose where the summary results should be saved. After choosing where the summary results should be saved the test will start **automatically**.

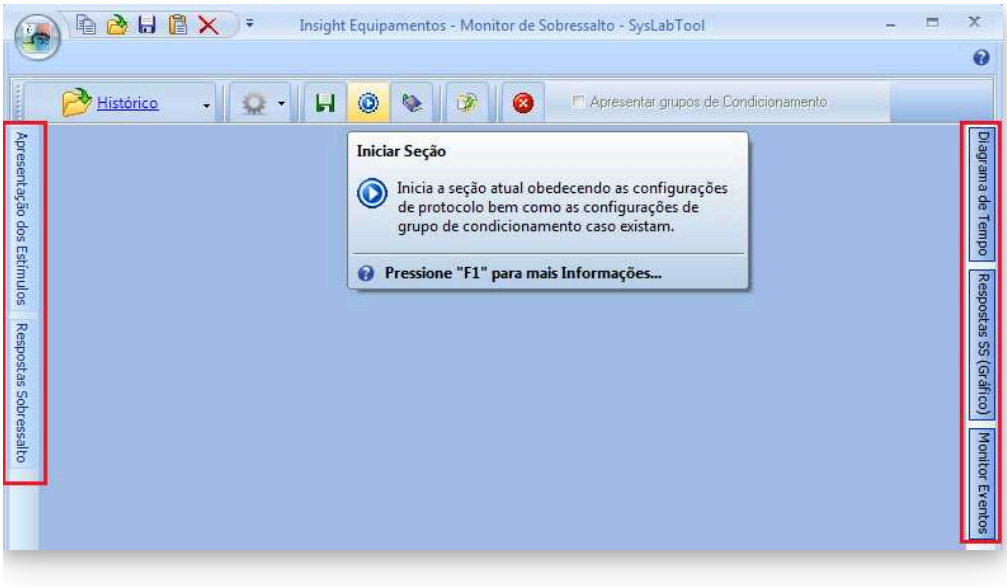



- If you set a habituation period, a new window will open and you should see the habituation countdown;



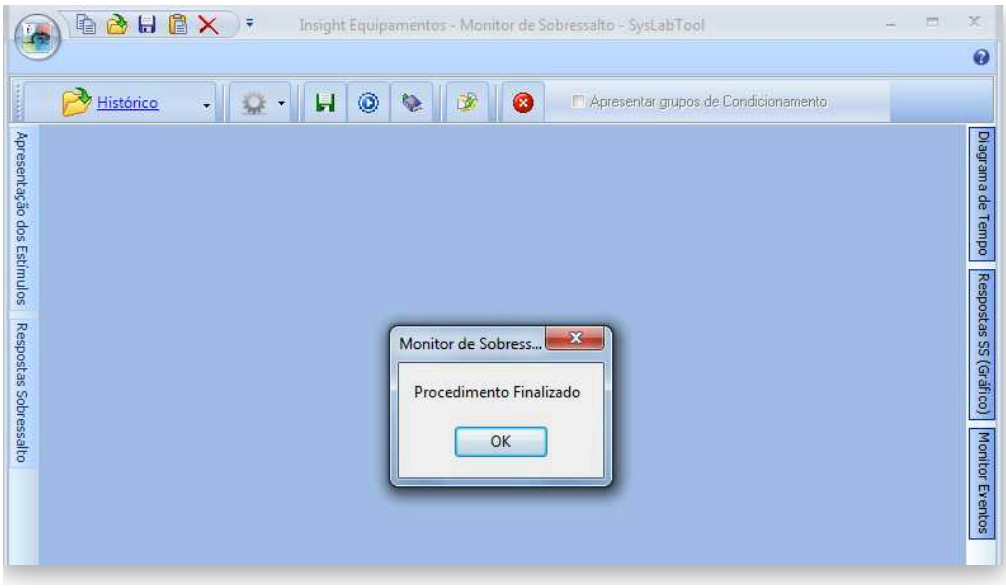

- When the habituation period is finished or if your protocol does not have habituation, the test will start;

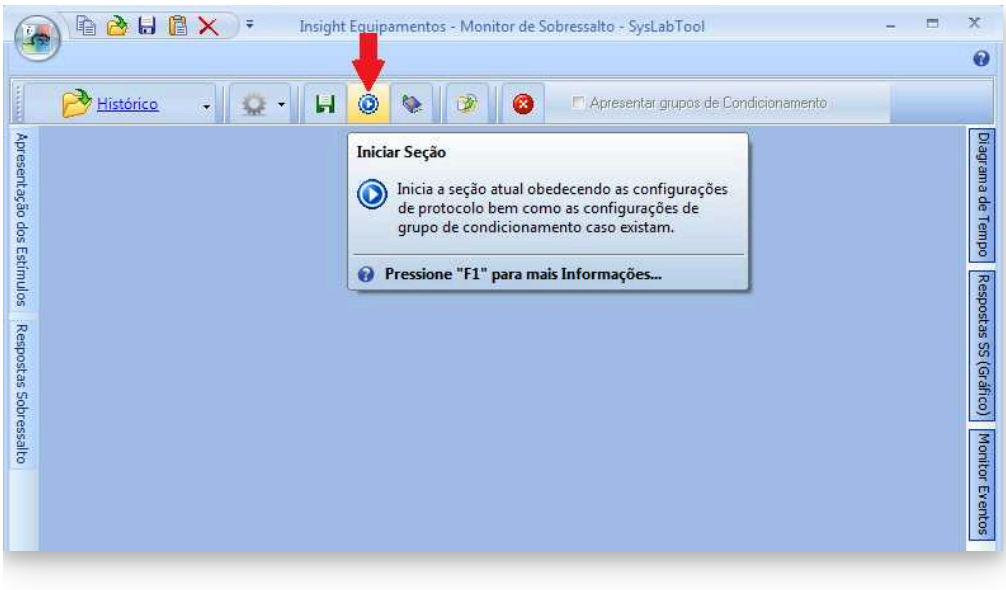

- After all trials run, the session will be finished and a window will pop up notifying you. During the test, you can click on any tabs on the main window to see the parameters collected.




13 **When the procedure is finished,** a new window will pop up. Click on "OK".

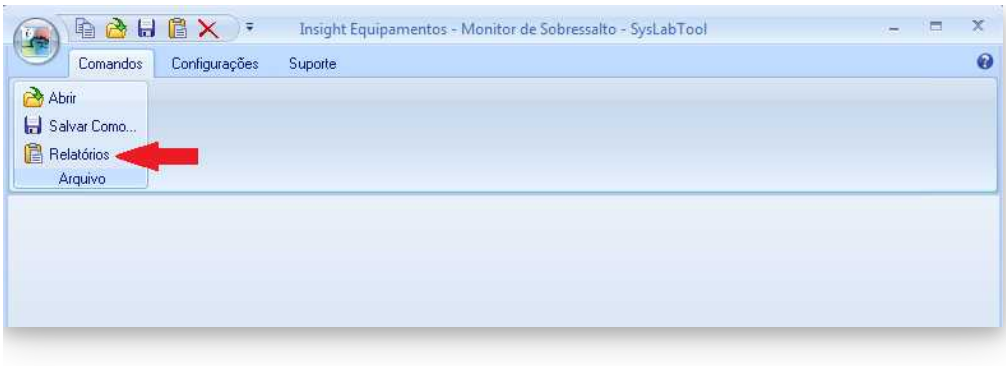
- **To start a new test**, place a new subject in the apparatus and click on the play button;
- There is no need to configure the protocol again, as the previous configurations are already saved;
- If the software displays an error message, close it and open it again. Open the protocol previously calibrated. There is no need to redo the calibration steps, you can use the same calibration parameters already saved. To do that, click next in the steps until you reach the last screen displayed on step 10. Click on the play button and start your test.



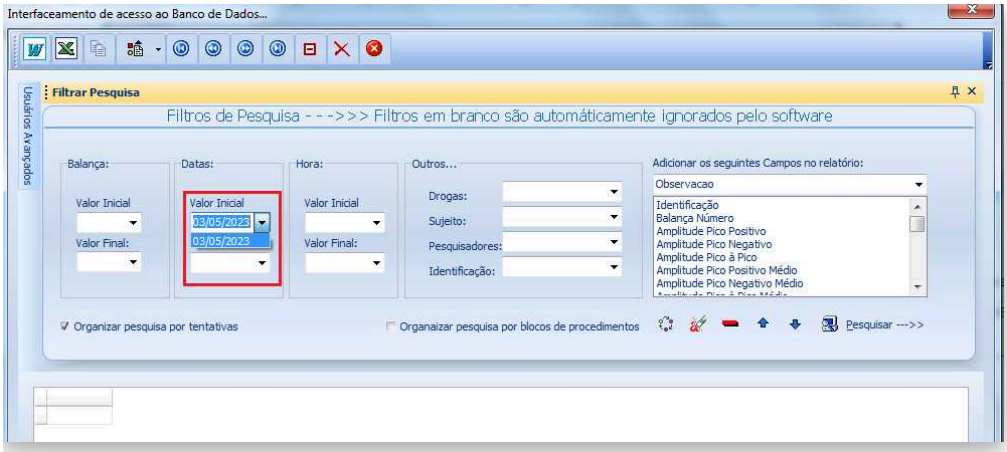




## Retrieving the data

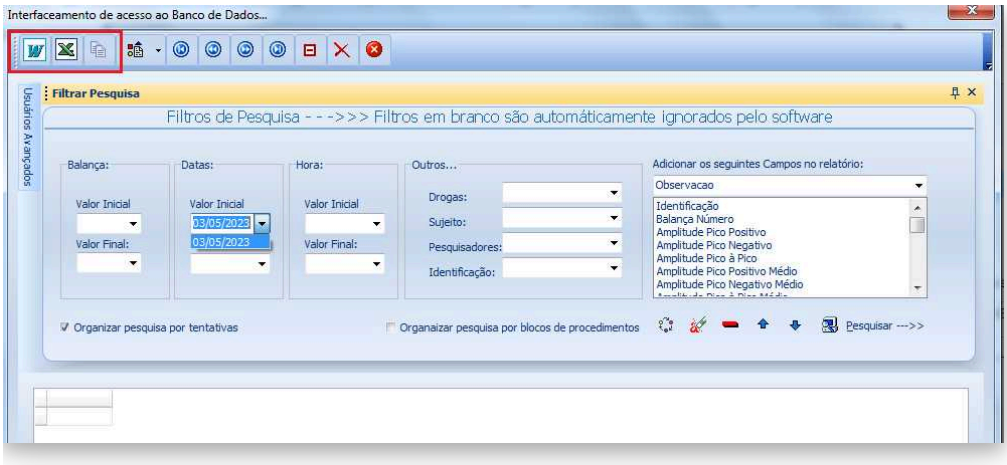
14 **When the test is finished or at any given moment that there is not a session in course,** you can export full reports by clicking on **"Reports"** on the main screen. A new window will open, and you will see numerous data filters available.




- You can use the parameters input in **step 8** go to step #8 to filter your data**.** We find date filters to be the more reliable parameters, but feel free to use as many filters as you find necessary;




- Once the desired data is displayed in the bottom half of the screen, you should click on one of the three icons in the top left of the screen - you can export data in **.xls**, **.doc** or **.txt**. This is the **raw data.**




## Data analysis

15 Data will be presented as arbitrary units. Important outcomes to be analyzed are the reactivity (startle) to all pulse and prepulse intensities and then prepulse inhibition for each combination, calculated as follow:

- For pulse and prepulse reactivity, use the **mean value of each stimulus**. For example, if pulse 100 dB was presented 10 times, you should use these 10 stimuli presentation values to discover pulse 100 dB **reactivity value**;
- For prepulse inhibition for each combination, use $(pulsealone - prepulse + pulse)/pulsealone * 100$. For example, if you want to calculate the PPI percentage for prepulse 71 dB combined with pulse 100 dB (pp71p100), you would use *(p100 - pp71p100)/p100 * 100;*
- Finally, for % mean PPI, use the mean value of all %PPI calculated for each combination of stimulus.

## Protocol references

For an example of a publication that followed this protocol see:
Franciele Kich Giongo, MSc, Matheus Gallas-Lopes, Radharani Benvenutti, PhD, Adrieli Sachett, PhD, Leonardo Marensi Bastos, Adriane Ribeiro Rosa, PhD, Ana Paula Herrmann, PhD, Effects of Taurine in Mice and Zebrafish Behavioral Assays With Translational Relevance to Schizophrenia,*International Journal of Neuropsychopharmacology*, Volume 26, Issue 2, February 2023, Pages 125–136,https://doi.org/10.1093/ijnp/pyac073