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## Psychophysics for a 2IFC task for multisensory comparisons

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<b>Corresponding Author:</b>	Luis Lemus Instituto de Fisiologia Celular Mexico, Mexico City MEXICO
<b>Corresponding Author's Institution:</b>	Instituto de Fisiologia Celular
<b>Corresponding Author E-Mail:</b>	lemus@correo.ifc.unam.mx
<b>Order of Authors:</b>	Fabiola Duarte Tonatiah Figueroa Luis Lemus
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**TITLE:**

A Two-interval Forced-choice Task for Multisensory Comparisons

**AUTHORS AND AFFILIATIONS:**

Fabiola Duarte<sup>1</sup>, Tonatiuh Figueroa<sup>1</sup>, Luis Lemus<sup>1</sup>

<sup>1</sup>Neurociencia Cognitiva, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico City, Mexico

**Corresponding Author:**

Luis Lemus (lemus@ifc.unam.mx)

Tel: +52 55 56225675

**E-mail Addresses of the Co-authors:**

Fabiola Duarte (fabiola.duarteortiz@gmail.com)

Tonatiuh Figueroa (tfigueroa@ifc.unam.mx)

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**SUMMARY:**

Psychophysics is essential for studying perception phenomena through sensory information. Here we present a protocol to perform a two-interval forced-choice task as implemented in a previous report on human psychophysics where participants estimated the duration of visual, auditory, or audiovisual intervals of aperiodic trains of pulses.

**ABSTRACT:**

We provide a procedure for a psychophysics experiment in humans based on a previously described paradigm aimed to characterize the perceptual duration of intervals within the range of milliseconds of visual, acoustic, and audiovisual aperiodic trains of six pulses. In this task, each of the trials consists of two consecutive intramodal intervals where the participants press the upward arrow key to report that the second stimulus lasted longer than the reference, or the downward arrow key to indicate otherwise. The analysis of the behavior results in psychometric functions of the probability of estimating the comparison stimulus to be longer than the reference, as a function of the comparison intervals. In conclusion, we advance a way of implementing standard programming software to create visual, acoustic, and audiovisual stimuli, and to generate a two-interval forced-choice (2IFC) task by delivering stimuli through noise-blocking headphones and a computer's monitor.

**INTRODUCTION:**

The purpose of this protocol is to convey a procedure for a standard experiment on psychophysics. Psychophysics is the study of perception phenomena through the measure of behavioral responses, elicited by sensory inputs<sup>1-3</sup>. Usually, human psychophysics is an inexpensive and essential tool to implement in imaging or neurophysiological experiments<sup>4</sup>.

However, it is never easy to select the most appropriate psychophysical method out of many that exist, and the selection somewhat depends on experience and preference. Nevertheless, we encourage beginners to revise available methodologies thoroughly in order to learn about selection criteria<sup>5-7</sup>. Here, we provide a procedure for performing a 2IFC task, which many researchers frequently use for studying perceptual processes such as working memory<sup>8</sup>, decision making<sup>9,10</sup>, or time perception<sup>11-13</sup>.

To guide the readers along the method, we recreate a report on the perceptual duration of visual (V), auditory (A), and audiovisual (AV) intervals of aperiodic sequences of pulses. We will refer to this task as an aperiodic interval discrimination (AID) task<sup>13</sup>. When attempting to describe this paradigm in psychophysics jargon, it would be a class-A, type-1, performance-based, criterion-dependent discrimination task that uses a non-adaptive method of the constants and a hyperbolic tangent (*tanh*) model to calculate a differential threshold. Even when such a characterization sounds somewhat entangled, we will use it to introduce the reader to some general aspects of psychophysics, hoping to provide decision criteria for new experiments and maybe even the possibility of tailoring the current protocol to other needs.

Any psychophysical experiment, such as a 2IFC task, requires implementing stimuli, a task, a method, an analysis, and a measurement<sup>6</sup>. The goal is to obtain the psychometric function that better accounts for the measured performance<sup>14</sup>. A 2IFC task consists of presenting to participants, who are naïve to the purpose of the experiment, trials of two sequential stimuli. After comparing the stimuli, they report the outcome by selecting one, and only one, out of two possible responses that better suits their perception.

With stimuli, we refer to technical considerations about the sensory modality under study. A class-A experiment consists of the comparison of stimuli of the same modality within a trial, whereas class-B experiments include cross-modal comparisons. Other essential considerations about stimuli include their implementation, such as the technical ways of modulating stimuli within a required range. For example, if we want to find the just noticeable difference (JND) between two flutter frequencies vibrating on the skin<sup>15</sup>, we need a precision stimulator to generate frequencies within the confines of flutter (*i.e.*, 4 - 40 Hz). In other words, the dynamic operating range of the technical elements depend on the dynamic spectrum of each sensory modality.

Selecting a task is about the perceptual phenomenon under study. For instance, finding whether two stimuli are the same, or equivalent, may rely on different brain mechanisms than those resolving if a stimulus is longer or shorter than a reference<sup>16</sup> (as in the AID paradigm). Intrinsically, stimuli selection defines the type of obtained responses. Type-1 experiments, sometimes closely related to the so-called performance experiments, include correct or incorrect responses. In contrast, a type-2 experiment (or appearance experiment) produces mostly qualitative answers that depend on the participant's criteria and not on any explicitly imposed criterion; in other words, criterion-independent experiments. It is noteworthy that 2IFC task responses are criterion dependent because, in every trial, the standard stimulus (sometimes called base or reference stimulus) constitutes the criterion on which the comparison's perception depends.

The method may refer to three things; first, it may refer to the mechanism for selecting the range of stimuli to test or, in other words, to an already known range of stimulus variability, as opposed to adaptive methods aimed to establish the adequate range<sup>17</sup>. These adaptive matters are recommended for quickly finding detection and discrimination thresholds and for minimum trial repetitions<sup>18</sup>. Also, adaptive methods are optimal for pilot experiments. The second definition of a method is the scale of stimuli modulations (*e.g.*, the method of the constants) or a logarithmic scale. The selected scale may or may not be a direct consequence of the outcome of an adaptive method, but primarily, it regards the dynamics of the studied sensory modality. Lastly, the method also refers to the number of trials and their presentation order.

As for analysis, it relates to the statistics of experimental measurements. Regardless of selecting appropriate analytical methods for comparisons between test and control groups, psychophysics is mostly about measuring absolute or differential thresholds between two conditions (*e.g.*, presence vs. absence of a stimulus, or the JND between two stimuli), particularly in 2IFC<sup>19</sup>. Such measurements derive from psychometric functions (*i.e.*, continuous models of behavior as a function of the probability of detecting or discerning one of the conditions at stake). Selecting the model function depends on the scale or, in other words, on the spacing of the values of the independent variable. Functions such as cumulative normal, logistic, Quick, and Weibull are appropriate for values spaced linearly, whereas Gumbel and log-Quick are better suited for logarithmic spacing. Alternative models also exist, such as the *tanh* employed in the AID task. Importantly, selecting a correct model depends on the parameters of interest, as considered in the design of the experiment<sup>20</sup>. After fitting the data to a model, it should be possible to derive two parameters:  $\alpha$  and  $\beta$  parameters. In the case of a logistic function typically employed in a 2IFC paradigm,  $\alpha$  refers to the abscissas value projecting to the point of subjective equality (*i.e.*, at half the logistic). The  $\beta$  parameter refers to the slope at  $\alpha$  value (*i.e.*, the steepness of the transition between conditions). Finally, a parameter commonly obtained out of a psychometric curve is the differential limen<sup>21</sup> (DL). In a 2IFC experiment, the DL relates to  $\beta$ , but strictly, corresponds to the minimum perceived difference between two intervals. The formula to determine the DL is the following equation (1).

$$DL = \frac{x_{0.75} - x_{0.25}}{2} \quad (1)$$

Here,  $x$  stands for independent variable values projecting at a 0.75 and 0.25 performance measured directly at the sigmoidal curve. Up until this point, we have covered only some generalities about psychometric functions. We recommend further study of estimating and interpreting psychometric functions, with these and other parameters<sup>22</sup>.

Other technical aspects to consider when implementing a psychophysical experiment are related to equipment and software. Memory and speed capacities of commercial computers nowadays are usually optimal for processing in high-fidelity visual and auditory tasks. Moreover, the dynamic resolution of complementary material, such as noise-blocking headphones, speakers, and monitors, must fulfill the sampling rate at which the sensory modalities operate (*e.g.*,

frequency, amplitude, contrast, and refreshing rate). Also, software programs such as PsychToolbox<sup>23</sup> and PsychoPy<sup>24</sup> are easy to implement and highly efficient at synchronizing tasks' events and equipment.

The previously described AID task assembles many of the topics described above for a 2IFC paradigm. Interestingly, it explores the perception of V, A, and AV intervals in the range of milliseconds, where most of the brain's processes occur<sup>25-27</sup>. Paradoxically, it is also a challenging lapse for studying vision, which, compared to audition, begets a somewhat constrained sampling rate<sup>28</sup>. In this sense, multimodal comparisons require additional theoretical scopes<sup>12,29,30</sup>. Sometimes, they need further tailoring to encompass a common modulation spectrum or to achieve congruent interpretations.

This protocol focuses on a discrimination task (*i.e.*, a 2IFC where a base stimulus, also called reference or standard, is contrasted against a set of comparison or test stimuli to find a JND or, in other words, a discrimination threshold). Here, the task is set to study the capacity of humans to discriminate time intervals of V, A, or AV aperiodic patterns of pulses<sup>13</sup>. We provide information on creating and parameterizing stimuli, as well as on analyses of accuracy and reaction times. Importantly, we discuss how to interpret subjects' time perception from the psychometrics statistical outcome parameters, and some experimental and analytical alternatives within topics of a 2IFC psychophysical method.

## **PROTOCOL:**

The experiments were approved by the Bioethical Committee of the Institute of Cellular Physiology of UNAM (No. CECB\_08) and carried out under the guidelines of The Code of Ethics of the World Medical Association.

### **1. Experimental Set-up**

#### **1.1. Material and stimuli set-up for performing an aperiodic interval discrimination (AID) task**

1.1.1. Perform this experiment on a computer with a minimum of 8 GB RAM, 2.5 GHz processor, and a 60 Hz refreshing rate monitor to create and run the task.

1.1.2. Obtain a set of noise-canceling headphones to avoid environmental sounds distracting the participants while performing the task.

1.1.3. Use a decibel meter to set the volume of the headphones to ~65 dB SPL.

1.1.4. Create the V, A, and AV stimuli for the task by running a graphical user interface (GUI) included in this protocol (**Figure 1**), or by using programs such as PsychToolbox or PsychoPy.

1.1.4.1. Download the Stimuli\_GUI.zip file from <http://www.ifc.unam.mx/investigadores/Luis-lemus>. Then, open MATLAB (2016a or higher for this GUI).

1.1.4.1.1. Click on the **SetPath** option at the MATLAB's menu tab to add the Stimuli\_GUI folder to the workspace. First, select the **Add Folder** button, select the **Stimuli\_GUI** folder, and press the **Save** button. Finally, close the window by clicking on the **Close** button.

1.1.4.1.2. Open the Stimuli\_GUI.m file using the **Open** option under the **Main** menu tab. Then, press **F5** on the keyboard to display the GUI (**Figure 1**).

1.1.4.2. Click on the **Condition popup** menu to select the preferred distribution of pulses (*i.e.*, Periodic for creating a stimulus of equidistant pulses, or Aperiodic for a random distribution). Then, select the desired number of pulses (*i.e.*, 2 - 6) in the **Number of Pulses popup** menu. Finally, enter the desired duration of the stimulus in the Duration dialog box.

CAUTION: To avoid V flicker fusion (*i.e.*, two or more pulses are perceived as only one), it is essential to create interpulse intervals (IPI) of a minimum of 30 ms. Therefore, given that each of the pulses is predefined to last 50 ms, the maximum number of pulses in a stimulus is constrained by the IPIs. Pulses exceeding the minimum IPI produce an error.

Note: The program generates images in an Audio Video Interleave (AVI) format at a rate of 60 frames per second. However, they can be created online at each trial using PsychToolbox or PsychoPy. Consider creating 50-ms pulses by concatenating at least three frames of 4° gray circles on black backgrounds. Here, the method generates AVI and WAV files for implementing in LabVIEW, thus suggesting the possibility of using more complex video or audio clips.

1.1.4.3. Click on the **Generate IPI** button to display the IPIs' values at the IPI values box and to see a plot of the resultant distribution of the pulses.

Note: The IPI values update automatically at every click of the **Generate IPI** button. These values can be copied and saved for further analysis.

1.1.4.4. Generate and store a V stimulus by typing a descriptive filename (*e.g.*, PeriodicVisual500ms.avi) in the Enter Video Filename dialog box. Click on the **Generate Video** button and wait for the popup window displaying gray ~4° circles to close. Then, click on the **Play** button to see the created V stimulus.

CAUTION: While the program generates the images, do not click on other figures, as this may cause the program to lose the figure's handle and produce a faulty video.

Note: The angular amplitude of a V object is obtained by the following equation (2).

$$V = 2 \tan^{-1}\left(\frac{S}{2D}\right) \quad (2)$$

Here,  $a$  is the amplitude of V expressed in degrees,  $S$  is the visual's size in centimeters, measured at the screen, and  $D$  is the distance in centimeters from the observer to the screen.

1.1.4.5. Generate and store an A stimulus using the same V IPI values by typing a descriptive filename (e.g., AperiodicAcoustic500ms.wav) in the Enter Audio Filename dialog box. Click on the **Generate Audio** button to observe a plot of the created audio and click on the **Play** button to listen to the new audio.

Note: The predefined A pulse frequency is 1 kHz; however, it is possible to change it in the Sound Frequency (Hz) dialog box.

1.1.4.6. Repeat steps 1.1.4.2 through 1.1.4.5 to create 10 aperiodic (AP) stimuli for each of the comparison intervals of the AID task (i.e., V and A intervals from 500 ms to 1,100 ms in steps of 100 ms). Create only one periodic (P) stimulus for each of the intervals of the control sets.

1.1.5. Generate an extended white-noise clip (e.g., 30 min) to use as background during the experiment, or download it from an internet library.

1.1.6. Create a 3° white cross and save it in a JPEG file to use as a cue for the participants to initiate a trial.

Note: AV stimuli result from superimposing V and A congruent clips during the execution of the task. Shift the A stimuli up to 90 ms after the V onset for producing perceptual simultaneity<sup>32</sup>.

## 1.2. Task design and implementation

1.2.1. Create sets of P and AP V, A, and AV trials by listing the names of the created stimuli in an Excel sheet. Use different columns to include all the information required during the task, such as the modality of the reference and comparison stimuli, the number of repetitions per trial, the stimuli durations, and the expected response (see an exemplary CSV file included in Stimuli\_GUI.zip file). Save each of the sets in a comma-separated values (CSV) format.

Note: The experiment aims to obtain psychometric functions of the probability of perceiving the test stimuli (i.e., the comparison stimuli) longer than the reference as a function of the variations of the comparison stimuli. Therefore, trials to generate psychometric functions must employ a reference stimulus fixed at half of the range of intervals (i.e., 800 ms). However, to guarantee that the participants' criteria rely on the reference stimulus, they must always attend to both the reference and the comparison. Therefore, trials of varying references should be included to counterbalance the number of varying comparisons. Finally, consider presenting blocks of V, A, and AV trials to avoid attentional effects. However, always present P and AP trials randomly intercalated.

1.2.2. Create a program for automatically running the task using PsychToolbox or PsychoPy, or download and run the automatized 2IFC\_Task available at <http://www.ifc.unam.mx/investigadores/Luis-lemus> (for running in LabVIEW 2014 or higher).

1.2.2.1. Open the 2IFC\_Task by double-clicking on the task's file.

1.2.2.2. Load the created stimuli by selecting the stimulus folder from the Control Panel. First, use the up and down buttons on the dialog box to display a 0. Then, press the folder icon to select the stimulus folder.

1.2.2.3. Repeat step 1.2.2.2 to set the File Path to 1, 2, 3, or 4 to load the CSV set of trials file, a TXT output file, a WAV background audio, and a cue of a white cross in JPEG format, respectively.

Note: When programming a task, store data in a convenient format for offline analysis (*e.g.*, in TXT or CSV format). Include information about the trial: the order of appearance and the behavioral outcomes, such as hits, errors, reaction times, and response times.

1.2.2.4. Press the **White Noise** button located on the Control Panel to activate the background noise. Then, place the decibel meter as near as possible to the headphones and set the OS volume control to ~65 dB SPL. Finally, adjust the **Background Volume** control located on the **Control Panel** to ~55 dB SPL.

1.2.2.5. Use the dialog boxes Pre\_S1 and Inter\_Stim to specify time-lapses of the first stimulus delivery and of the interstimulus separation, respectively.

Note: The default times are 1,000 ms. Other graphical indicators are for the examiner to observe results in real-time (*e.g.*, a bar plot of the performance per condition and displays of the number of hits, errors, false alarms, and a current number of trials).

1.2.2.6. Test the task by clicking on the **Run** right arrow icon under the **Tools** tab and perform some test trials.

Note: We recommend to use two monitors, one for delivering the task and the other for monitoring the task online.

1.2.2.6.1. Initiate each trial by holding the spacebar down after the appearance of the visual cue at the center of the screen. Release the spacebar after the delivery of a pair of stimuli and press the upward or the downward arrow key to finalize the trial.

1.2.2.6.2. Repeat step 1.2.2.6.1 until the set is completed. The task stops automatically. Alternatively, abort the task by clicking on the **Stop** button in the **Control Panel** menu.

## 2. Participants

2.1. Recruit 10 to 30 male and female right-handed participants, with no more than ten years of difference in age between them, with normal or corrected-to-normal vision, and no auditory deficits.



2.2. Ask the participants to fill out a questionnaire regarding their age, gender, handedness, and physical or psychological conditions (e.g., having visual or auditory deficits, musical training, and drug intake).

2.3. Tell the participants about the aim, procedures, and duration of the experiment. Be careful no bias is induced (e.g., telling them about the occurrence of P or AP conditions). Then, ask the participants to give written consent to participate in the experiments.

### 3. Experimental Procedure

3.1. Perform the experiments in a quiet room with constant lighting.

3.2. Run the task.

3.3. Calibrate the decibel meter and repeat the procedure as described in step 1.2.2.4.

CAUTION: Throughout the experiment, acoustic stimuli should be delivered binaurally at ~65 dB SPL. It is essential to use a decibel meter to test the acoustic amplitudes before the experiment to avoid injuries.

3.4. Ask the participant to sit comfortably in front of the monitor, situated at a distance of 60 cm. Then, place the keyboard at a reachable distance, and adjust the headphones to the participant's head (Figure 2C).

3.5. Instruct the participant to start a trial after the appearance of the visual cue by pressing and holding down the spacebar for the entire trial. Indicate to the participant to release the spacebar after the presentation of two sequential stimuli and to press the upward arrow key if the second stimulus lasted longer than the first, or to press the downward arrow key if it lasted for a shorter period of time (Figure 2B).

3.6. Finally, instruct the participant to use only the right index finger to complete the task, and comment on the possibility of taking a 5-min break in case the participant feels tired or distracted during the experiment.

3.7. Turn on the noise-blocking feature of the headphones and let the participant practice 10 - 15 trials.

Note: During this phase, providing a visual input for correct answers is recommended. Also, it is possible to provide feedback during the experiment; however, be aware of possible biases.

3.8. Run the task.

### 4. Data Analysis

4.1. Calculate the mean and the standard error of the mean of the performance of each of the blocks of P and AP V, A, and AV trials.

4.2. Generate scatter plots of the probability of perceiving the comparison stimulus longer than the reference as a function of the comparison's intervals. Then, fit a logistic function to the data.

Note: As noted in the **Introduction**, selecting a convenient model depends on the experiment and the data. An example of a model is the *tanh* as reported for the AID task. Such a model delivers four parameters (inset in **Figure 3A**) defined by:

$$P(S1 > S2) = a \times \tanh[\beta(s2 - \theta)] + c$$

The parameter **a** corresponds to the performance's magnitude measured from the inflection point to the plateau. The **β** parameter corresponds to the first derivative at the inflection point. The higher the value, the easier to perceive a transition between longer and shorter, when compared to the reference categories. The parameter **θ** or X0 is the abscissa value of the projection of the inflection point (*i.e.*, the point of subjective equality). The shifting of such a parameter represents overall temporal biases. Finally, **c** or Y0 represents the inflection point at the ordinate and reveals biases toward a particular response. Alternative routines for fitting and analyzing psychometric functions are the Palamedes toolbox<sup>6</sup> and quickpsy<sup>33</sup>.

4.3. Repeat the procedure from step 4.1 for analyzing the reaction times and response times.

4.4. Perform statistical analyses to compare the P and AP accuracy distributions within each of the sensory modalities.

4.5. Perform additional analyses, such as Pearson correlations, to find the relationship between periodicity indices and accuracy and between periodicity indices and reaction times.

## REPRESENTATIVE RESULTS:

This protocol presented a method to perform a psychophysics experiment in humans. The technique replicated previous research on the discrimination of intervals of AP trains of V, A, and AV pulses, which was performed using a 2IFC method. The stimuli resulted from P and AP distributions of trains of six 50-ms pulses in different intervals within the range of milliseconds (*i.e.*, from 500 ms to 1,100 ms in steps of 100 ms). **Figure 2A** shows some intervals and their calculated periodicity index.

The task was programmed in LabVIEW and consisted of delivering intramodal trials of two sequential stimuli (**Figure 2B**). After 31 participants (15 women and 16 men of an age of  $23.6 \pm 4.3$  years [mean  $\pm$  standard deviation]) performed the task, we obtained the psychometric functions of each of the P and AP V, A, and AV overall accuracies using a *tanh* function (**Figures 3A - 3C**; goodness of fit:  $\chi^2$ ,  $Q > 0.05$ ).

The right panels in **Figure 3** show comparisons of the regression parameters of the *tanh* to the P and AP conditions. Non-overlapping variances of such parameters indicated statistical differences, for example in V's  $\alpha$ ,  $\beta$ , and  $c$  values ( $p < 0.05$ ). This result is apparent at the V AP sigmoid shifting downward, suggesting that the participants perceived the intervals longer than the reference as shorter (**Figure 3A**). Similarly, intervals that were shorter than the reference were accurately regarded as shorter since the  $c$  parameter showed an AP shifting to a probability of calling the comparison shorter than the reference. Moreover, comparisons of AP and P intervals during A and AV discriminations showed differences in  $\beta$  and  $\theta$  parameters ( $p < 0.05$ ) because the overall AP accuracies decreased, suggesting that the AP stimuli were generally harder to discriminate. Interestingly, the A and AV performances were similar during P and AP conditions (**Figures 3B** and **3C**), indicating an A dominance over V in AV discriminations.

In the V condition,  $\beta$  proved that the transition from shorter to longer occurred faster in the AP condition. This result suggests that the participants were confident of their decisions, as evidenced by the reaction times (**Figure 4A**). In contrast, reaction times of P and AP AV resemble those of the A conditions, also suggesting an A dominance (**Figures 4B** and **4C**). The overall interpretation of these results is that V AP patterns produced a perceptual compression of the V AP time intervals.

The psychophysical results reflect differences in information processing across sensory modalities. When we asked the subjects to discriminate between the duration of AP patterns, we found that the modality alters the time perception differentially. The V system compresses the estimation of time, whereas A and AV accuracies were only slightly affected by AP structures. Altogether, these results show different examples of interpreting the outcomes of a psychophysical task through their psychometric parameters.

## FIGURE AND TABLE LEGENDS:

**Figure 1: A graphical user interface (GUI) to create AID's task stimuli.** The GUI allows creating a visual or an auditory stimulus by introducing parameters and naming the stimulus. A graphical representation of the resulting interpulse intervals (IPI) and a plot of the auditory stimulus is shown in the windows on the right. A thorough description of how to implement this GUI is described in the text.

**Figure 2: Task structure and set-up. (A)** This panel shows a graphical depiction of intervals of different durations of aperiodic trains of pulses represented by gray squares. The intervals and their periodicity indices obtained by atop formula are shown in different lines and as a function of their durations. PI = periodicity index; P = periodic; AP = aperiodic. **(B)** This panel shows the sequence of events during unimodal trials. Each of the trials started when a participant released the spacebar (SBD). After a reference stimulus followed by a 1-s interstimulus, a comparison stimulus was delivered, the participant released the spacebar (SBR), and, in order to report whether the comparison was longer or shorter than the reference, pressed the upward or the downward arrow key, respectively (choice). The reference and comparison's gray squares represent pulses of actual visual, acoustic, and audio-visual pulses depicted by icons above. **(C)**

This panel shows the depiction of the experimental set-up. The material includes a computer, a set of noise-canceling headphones, a monitor, and a keyboard. **Figures 1A and 1B** are adapted from Duarte and Lemus<sup>13</sup>, under the guidance with the Copyright Statements of Frontiers in Integrative Neuroscience.

**Figure 3: Psychometric functions obtained by the hyperbolic tangent regression model.** (A) This panel shows the overall probabilities of declaring comparisons longer than the reference as a function of the comparison's time intervals of the visual periodic (solid lines) and visual aperiodic (dotted lines) experiments. (B) This panel shows the same information as panel A, but then for acoustic conditions. (C) This panel shows the same information as panel A, but then for audiovisual conditions. The right panels show the distribution of the *tanh* parameters as defined in the inset in panel A. The error bars in panels A - C denote the standard error of the mean and the confidence intervals in the right panels. The asterisks express intramodal differences. P = periodic; AP = aperiodic; V = visual; A = acoustic; AV = audiovisual. This figure is modified from Duarte and Lemus<sup>13</sup>, in guidance with the Copyright Statements of Frontiers in Integrative Neuroscience.

**Figure 4: Reaction times.** (A) This panel shows the mean reaction times for visual experiments. (B) This panel shows the mean reaction times for acoustic experiments. (C) This panel shows the mean reaction times for audiovisual experiments. Solid lines = periodic intervals. Dashed lines = aperiodic intervals. The error bars denote the standard error of the mean. This figure is modified from Duarte and Lemus<sup>13</sup>, in guidance with the Copyright Statements of Frontiers in Integrative Neuroscience.

**Table 1: List of materials.**

## DISCUSSION:

In psychophysics, the selection of a task depends on particular interests in perceptual phenomena<sup>5,6</sup>. For instance, this protocol consisted of recreating a previously reported paradigm on the time-interval perception of visual, auditory, and audiovisual stimuli of aperiodically arrayed pulses, which implemented the 2IFC method<sup>13</sup>. Here, as in most of the psychophysics tasks, adequate hardware and software are essential to create, reproduce, and record the task's elements accurately, especially when exploring phenomena that occur in the range of milliseconds<sup>25-27</sup>. An advantage of the current method is the ability to produce diverse stimuli through a GUI since it allows exploring their metrics and performance. In this respect, it is worth bearing the importance of the parameterizing variables regardless of the complexity of the stimuli, as in this protocol, which implemented a simple but novel method for quantifying aperiodicity<sup>13</sup> (**Figure 2A**). We also proposed storing the stimuli in audio and video formats such as WAV and AVI because that presents the possibility of implementing large video clips in experiments. However, those formats require administering hardware and software processing efficiently; for instance, by prebuffering stimuli into variables of the environment of the conducting program. Notwithstanding, some programs such as PsychToolbox or PsychoPy are useful for, alternatively, creating online stimuli.

Although we did not include the results of pilot experiments, it is advisable to perform them in order to check the correct functioning of the equipment and to find the satisfactory range and scaling of the independent variables<sup>18,34</sup>. In this sense, implementing adaptive psychophysical methods is recommended<sup>6,17</sup>. Moreover, pilot experiments determine the adequate pool of participants and the number of trial repetitions, thus yielding robust results and statistical analyses<sup>14</sup>.

With regard to the participants, it is always important to clearly instruct them as to what they should attend and how they should perform. Otherwise, adopting alternative strategies could mislead the results<sup>21,35</sup>. For example, in this task, we asked the participants to discriminate the duration of the stimuli; however, typical behaviors include discriminating speed, acceleration<sup>29</sup>, the number of events<sup>11</sup>, or reporting similarities. In other words, while it is possible to observe similar performances among participants, results may yet be flawed by including different brain processes<sup>16</sup>. Therefore, together with adequately instructing the participants, it is mandatory to ask them about their adopted strategy for solving the task.

An inherent problem of psychophysics comes from the nature of sensory modalities since they impose limits on methodologies<sup>12,29,30,32</sup>. For example, given that visual frames delivered above 15 Hz are likely to create flicker fusion<sup>28</sup>, studying the visual perception of pulses requires slow modulations to avoid undesired results. Furthermore, comparisons among sensory modalities escalate the problem. In this regard, an interesting phenomenon observed in the AID experiment was that aperiodic visual stimuli created a perceptual compression of temporal estimations but the periodic ones did not. There, the *tanh* function fitted to the data optimally because the observed aperiodic visual plateau did not reach a maximum probability of 1, such as other logistic models predict (**Figure 3A**). However, regardless of selecting the best logistic model, it could be argued that the aperiodic visual did not reach a probability maximum because the stimuli range was insufficient. Therefore, increasing the duration of the intervals or decreasing the number of pulses would probably produce a different result<sup>17</sup>. However, there is a much more profound issue here that actually relates to a dilemma in psychophysics. First, the AID experiment aimed to test the interval perception in the range of hundreds of milliseconds, which accounts for a particular case of temporal processing<sup>26,27</sup>. Therefore, increasing the interval durations would result in testing a different brain mechanism<sup>16</sup>. Second, the visual periodic control proved to operate within an adequate range; hence, spreading visual intervals was not justified. Finally, adjusting one of the condition's intervals alone disables comparisons across groups or, importantly here, between sensory modalities<sup>30</sup>. Again, adapting auditory and audiovisual intervals were not justified (**Figures 3B and 3C**). Thus, the dilemma is that aiming to obtain perfect psychophysical distributions may mix neuronal processes, whereas not doing so may produce suboptimal results.

In conclusion, psychophysics consists of studying the behavioral outcome of neuronal mechanisms of sensory processing. Such a challenging goal demands the optimal selection and implementation of the stimuli, the task, the method, the analysis, and the measurement<sup>6</sup>. When mastering psychophysics, it provides valuable insights into perception. Furthermore, it is

essential in models that require well-trained animals for studying, for example, the neurophysiological correlation of behavior<sup>10,30,36,37</sup>.

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The authors have nothing to disclose.

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Figure 1

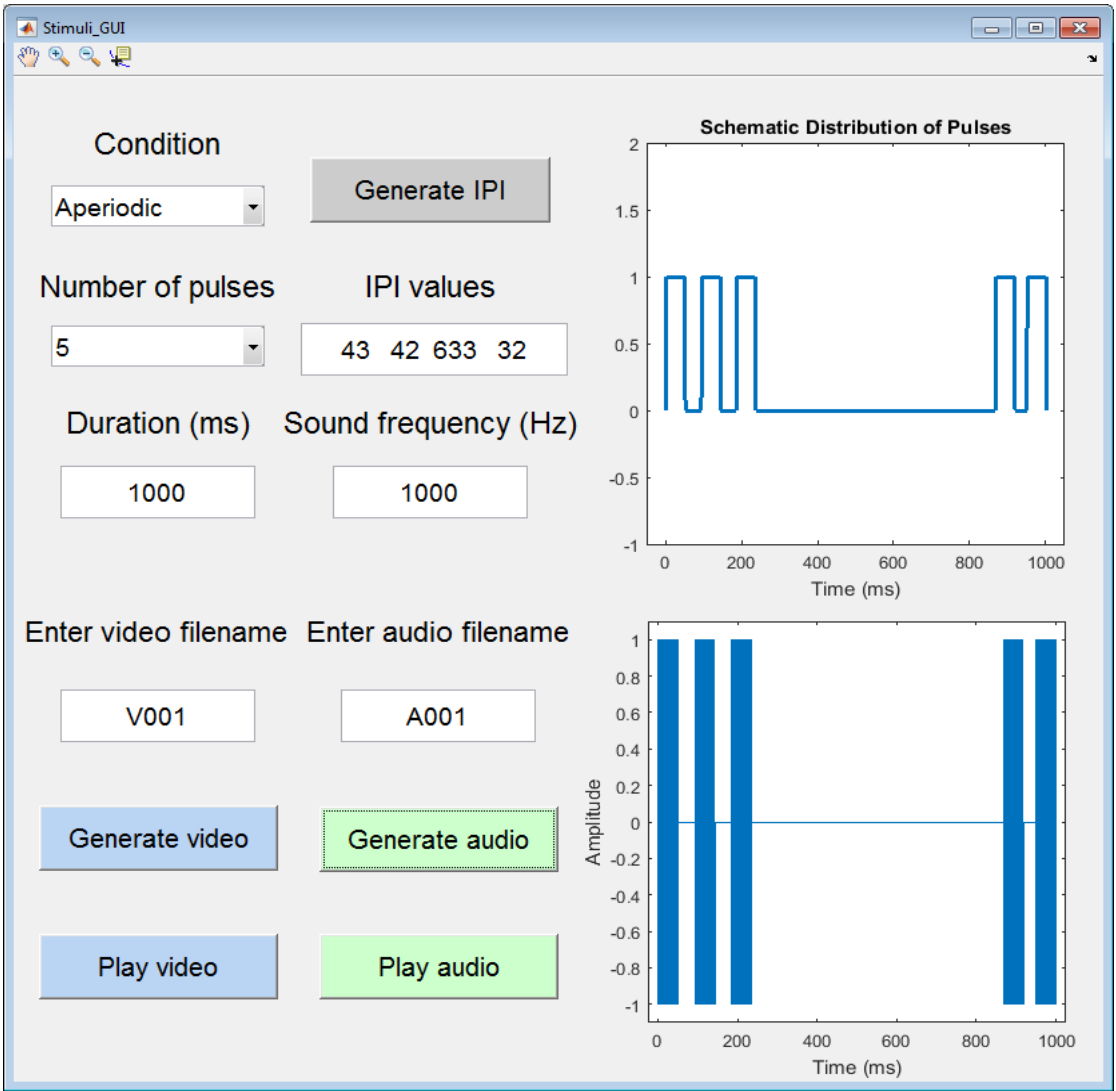


Figure 2

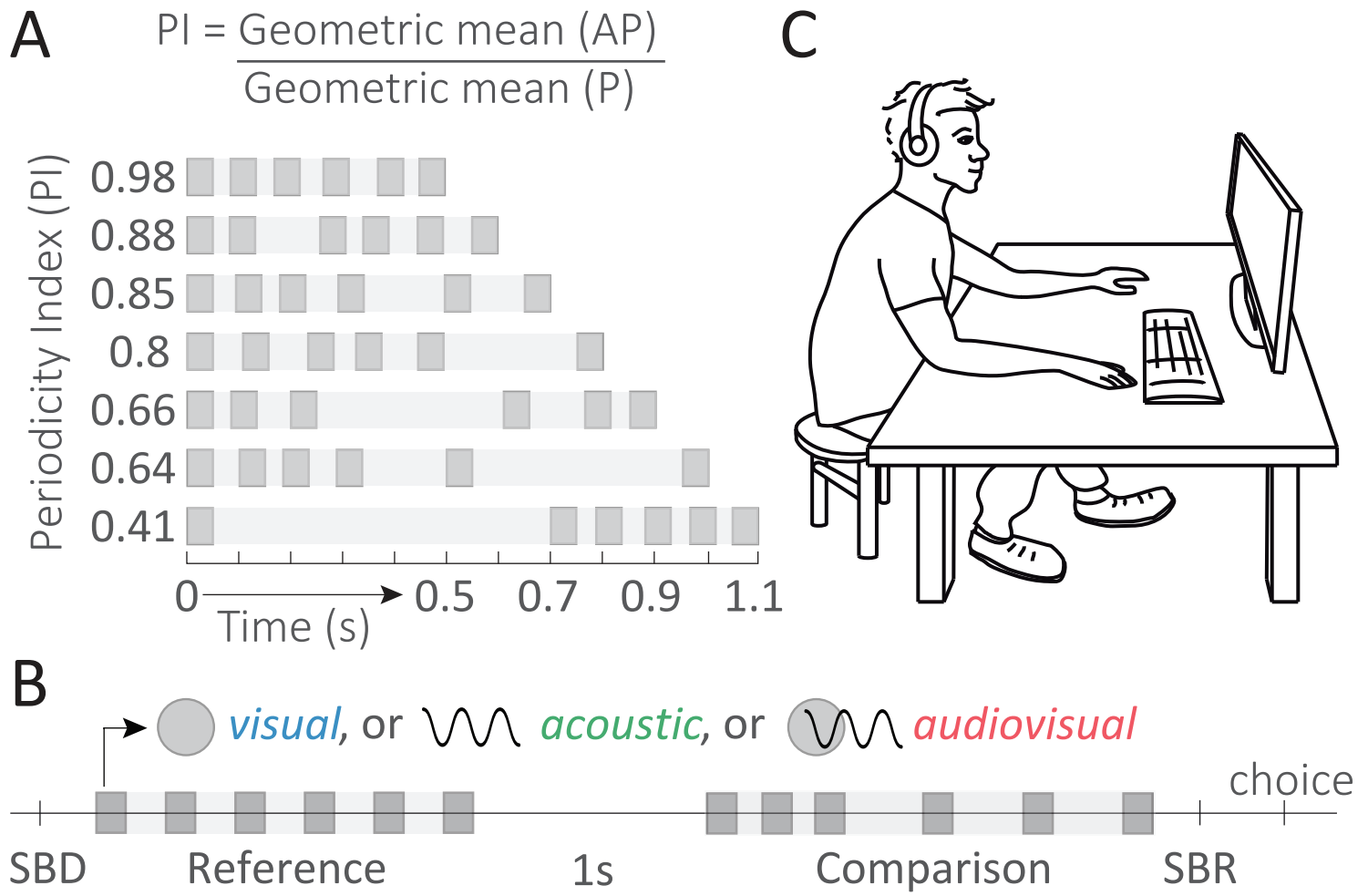


Figure 3

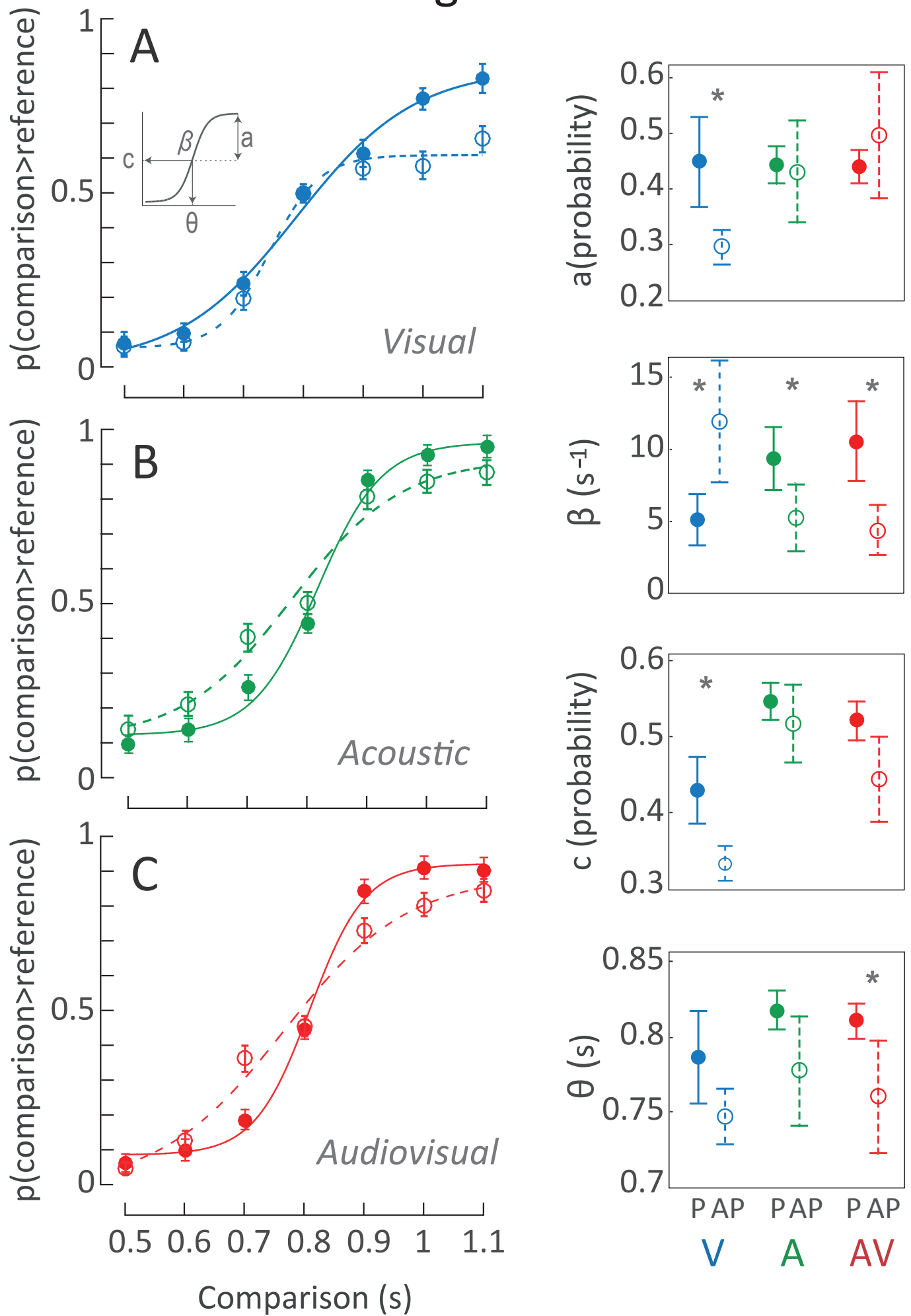
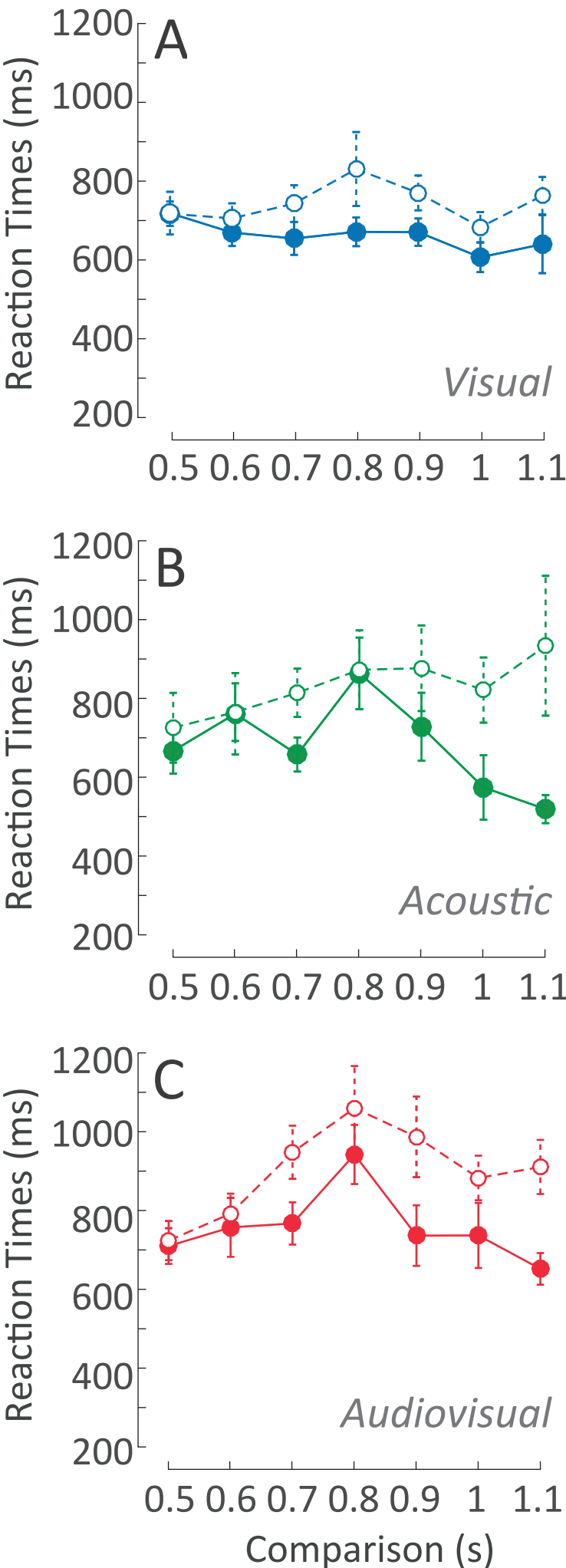


Figure 4



<b>Name of Material/ Equipment</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments/Description</b>
Lapt top Dell Precision	Dell	M6800 CTO	Procesador Intel Core i7-4710MQ, 2.5GHz RAM 16 GB, 64-k
Noise-blocking headphones	Bose	QC25	Headphones QuietComfort 25, noise-blocking
Decibel meter	Extech Instruments	SL 130G	Sound Level meter (dB), range 30 to 130 dB, this meter me

<b>Software</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments/Description</b>
Labview	National Instruments	Labview 2014	Labview SP1 130, 64-bits, version 14
Matlab	Mathworks Inc	Matlab 2016a	The Mathworks Inc., Natick, MA, USA
GUI To create Visual and Acoustic stimuli. Created by Fabiola Duarte	Mathworks Inc	Matlab 2016a	The Mathworks Inc., Natick, MA, USA

bit OS; 17.3" screen 1920 x 1080; 60 Hz refreshing rate

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Luis Lemus

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Cognitive Neuroscience

Institution:

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