

A New Computational Tool for the Post Session Analysis of the Prepulse Inhibition Test in Neurscience

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Abstract

Prepulse inhibition (PPI) refers to the reduction in startle reaction towards a startle-eliciting “pulse” stimulus when it is shortly preceded by a sub-threshold “prepulse” stimulus. PPI deficits have been seen in various neuropsychiatric disorders, such as schizophrenia, Tourette’s syndrome, and Huntington’s disease. Recent animal studies have employed PPI test to address issues relevant to mental disorders. Measuring the acoustic startle reflex and calculating PPI in small animals produces myriads of numeral data. These raw data need to be justified and organized properly before being analyzed statistically. Therefore, organizing and analyzing these raw data without a computer software is time consuming and tedious. The software we created is useful and powerful in the post session data analysis of PPI test as it has the following three advantages: (1) grouping data under different chambers and trials; (2) eliminating questionable data; (3) batch processing data, which enable researchers to finish the post session data analysis for a number of PPI tests in a few seconds.

1. Introduction

1.1 The definition of prepulse inhibition

Prepulse inhibition (PPI) refers to the reduction in startle reaction towards a startle-eliciting “pulse” stimulus when it is shortly preceded by a sub-threshold “prepulse” stimulus [1; **Figure 1**]. The startle reflex is a constellation of responses to sudden, relatively intense stimuli. In humans, the blink reflex component of startle is most often measured using electromyography of the orbicularis oculi muscle. In

rats and mice, the whole-body flinch in response to acoustic stimuli can be measured.

PPI is thought to result from the activation of brain mechanisms by which an organism filters information from its surroundings. PPI is significantly reduced in specific neuropsychiatric disorders characterized by symptoms associated with central inhibitory deficits, including schizophrenia, Tourette’s syndrome, and Huntington’s disease [2-5]. Also, PPI can be disrupted by systemic apomorphine in animals [6].

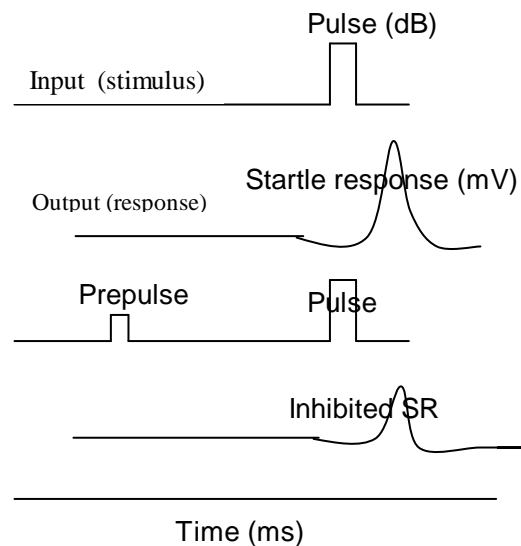


Figure 1. Prepulse inhibition of the acoustic startle reflex.

1.2. The SR-LAB system and measurement of startle response

Startle in laboratory animals is usually measured by recording whole body startle, which is taken for about 200 milliseconds (ms) after presentation of the

eliciting stimulus. Of the existing measuring systems, the SR-LAB system is a preferential one, which is an automated system for measuring startle responses of small animals. This system is composed of four major units: the Control Unit (CU), the Startle Chamber/Isolation Cabinet (SC), the Animal Enclosure (AE) and the Data Acquisition Board (DAB; or card) for the desktop (or laptop) computer. The CU provides the connection for up to 16 startle chambers. The SC is designed to provide sound attenuation and visual isolation of the test subject. Each chamber includes light and fan controls. A speaker, mounted 24 cm above the animal, provides background noise, prepulse stimuli and startle stimuli, all controlled by the SR-LAB software. The AE is designed to locate the test animal so that the body of the animal is centered over the motion sensor (piezoelectric sensor), in which the animal's movements create an electrical current. The DAB digitizes the electrical signals to numerical values which are stored by the computer (Figure 2).

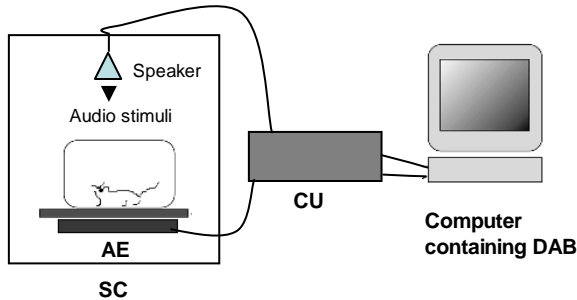


Figure 2. The hardware of SR-LAB system measuring startle response. AE, animal enclosure; CU, control unit; DAB, data acquisition board; SC, startle chamber.

In a recent study, we performed PPI tests using C57BL/6 mice as subjects [7]. Briefly, each subject was placed in the startle chamber for five minutes to allow the subject to adapt to the test enclosure before starting a PPI test. Each test session started and ended with 5 startle trials, respectively. These startle trials were not included for the post-session analysis. Between the starting and ending startle trials, there were 8 identical blocks; each consists of the following 5 trials: a no-stimulus trial, a startle trial, and three prepulse-startle trials, each of which had a pre-pulse stimulus (3, 6, or 12 dB above the background sound levels) prior to a startle stimulus (100 ms after the prepulse). These trials were ordered randomly and separated by inter-trial intervals (ITI) with a range of 8 to 23 seconds. Using a variable ITI is intended to minimize habituation of startle across the trials.

Measures were taken of the startle amplitude for each trial, defined as the peak response during a 65-ms sampling window starting from the onset of a startle stimulus. The outline of a typical startle test block is shown in Figure 3.

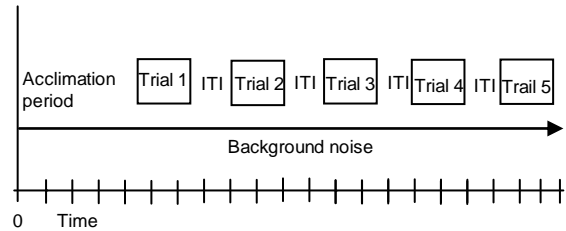
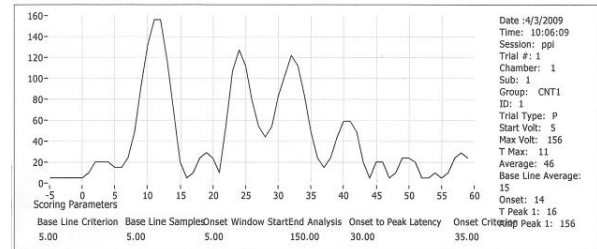


Figure 3. The outline of a typical startle test block

2. The post session analysis of the startle response

2.1 Data reporting with the SR-LAB system [8]

Figure 4 is a typical startle response graph shown on the post session analysis window.



The horizontal axis of the graph is the number of record samples (ms). The vertical axis represents the amplitude of the startle response in millivolts. As shown on the window, the startle response is described by the following parameters:

Start (mV): The voltage at the start of the response window.

V_{max} (mV): The highest voltage during the response window, or the “peak” of the response.

T_{max} (ms): The time in milliseconds at which the V_{max} appear after the start of the response window. In other words, this is the “latency” to the “peak” of the response.

Avg. (mV): The average voltage across the entire response window.

Baseline (mV): The average voltage of five samples following the start analysis parameter specification. It informs the user if there is any excessive activity prior to the stimulus.

T-Peak 1 (ms): The time at which the first peak appears.

Amp P1 (mV): The amplitude of the first peak.

2.2 Data analysis of the startle response needs powerful software

Although the SR-LAB system has the functions of reviewing and concatenating data, the data analysis must be done by researchers. As described above, each PPI test session consists of a number of blocks, each block is composed of several trials, and the response graph of each trial is characterized by several parameters as defined above. In practice, usually there are several chambers connected to a control unit. After finishing PPI tests, the data from individual chambers are usually concatenated and these raw data are lined randomly as shown in **Figure 5**. Moreover, all of the raw data need to be examined and justified carefully as certain trials may contain questionable data that should be excluded from being statistical analysis. For example, the parameter **Start** is intended only as a “stability” check, which can signal that the subject, before responding to a startle stimulus, was moving, or making other motions that might interfere with the proper recording of a startle response. Thus, including a trial for statistical analysis is not proper if the value of the **Start** in this trial is high (close or greater than the **Avg**) [8].

Therefore, analyzing the raw data is time consuming and tedious. It should be performed automatically by a computer program. But, at our hands, no this kind of software was available.

Date	Time	Session	Subject	Group	ID	Trial	Trial type	Chan.	Seq.	Start	V Max	I Max	Avg	
4/3/2009	10:06:09	ppi		1	CNT1	1	1P		1	1	5	166	16	42
4/3/2009	10:06:09	ppi		1	CP21	1	1P		1	2	0	54	31	19
4/3/2009	10:06:09	ppi		1	CNT1	1	2P		1	1	5	405	17	118
4/3/2009	10:06:09	ppi		1	CP21	1	20P		1	2	24	54	54	20
4/3/2009	10:06:09	ppi		1	CNT1	1	21 pP11		1	1	5	49	53	19
4/3/2009	10:06:09	ppi		1	CP21	1	21 pP11		1	2	10	20	32	8
4/3/2009	10:06:09	ppi		1	CNT1	1	22 pp22		1	1	5	88	29	33
4/3/2009	10:06:09	ppi		1	CP21	1	22 pp22		1	2	5	54	30	14
4/3/2009	10:06:09	ppi		1	CNT1	1	23 pP33		1	1	0	15	6	4
4/3/2009	10:06:09	ppi		1	CP21	1	23 pP33		1	2	15	29	48	11
4/3/2009	10:06:09	ppi		1	CNT1	1	24 P		1	1	5	552	17	130
4/3/2009	10:06:09	ppi		1	CP21	1	24 P		1	2	5	68	52	18

Figure 5. The raw data of PPI test reported by the SR-LAB system. The highlighted rows contain questionable data.

3. The new post session data analysis software

3.1 Objectives

(1) Grouping data under different chambers and trials.

(2) Eliminating the starting and ending trails as well as the questionable data. As described before, the starting and ending pulse trials are to serve to habituate and stabilize the animals’ startle response [6]. In addition, inevitably, each test session contains certain questionable data resulted from other motions unrelated to the startle stimulus. The trials containing these questionable data must be eliminated from statistical analysis. In our practice, two criteria were pre-defined as: **Start < Avg and T_{max} < 52 ms**.

(3) Performing batch processing for a number of files at one time. It means all raw data from an experiment can be done in a few seconds at one time; it is not necessary to analyze the raw data from each test session individually.

3.2 Implementation of the post session data analysis software

To achieve the above objectives and automatically execute the whole process, we created a program PPI post session analysis (PPI-PSA). The efficiency of this program is highly expected since we have enormous data from each experiment. Therefore, a c++ runtime

library was used. A simple interactive user interface was provided to receive user's commands, such as whether it needs to perform the questionable data elimination or not. The PPI of each test session was calculated according to the following formula:

$$\text{PPI} = \frac{\text{SR without prepulse} - \text{SR with prepulse}}{\text{SR without prepulse}} \times 100$$

Where, SR stands for the startle response.

The program can check and do batch processing for all input files within a designated folder named as "input_data". All statistical results would be output to a folder "output_data". For example, inputting the file PPI_CU_CPZ_04012009.xls to "input_data", and running the program PPI-PSA, in a few seconds, the statistical data of all parameters, after eliminating questionable data, will be provided and saved in a file PPI_CU_CPZ_04012009_output.xls within the folder "output_data". The flowchart of the PPI-PSA is illustrated in **Figure 6**.

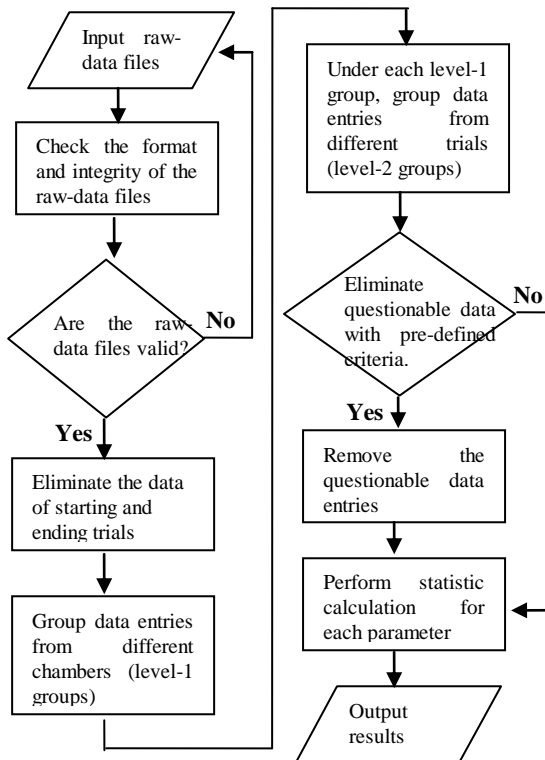


Figure 6. The flowchart of the PPI post-session analysis software. * the pre-defined criteria are: Start < Avg; T_{max} < 52 ms.

4. Concluding remarks

PPI test offers an operational measure of sensory gating of subjects. PPI has also been extensively employed in animal studies addressing issues relevant to mental disorders, especially schizophrenia which is a complex and severe brain disorder. Measuring the acoustic startle reflex and calculating PPI in small animals produces myriads of numeral data. These raw data need to be justified and organized properly before being analyzed statistically. Therefore, organizing and analyzing these raw data without a computer software is time consuming and tedious. The software we created is useful and powerful in the post session data analysis of PPI test as it has the following three advantages: (1) grouping data under different chambers and trials; (2) eliminating questionable data; (3) batch processing data, which enable researchers to finish the post session data analysis for a number of PPI tests in a few seconds.

Acknowledgements

All data came from the animal studies, which were performed in the lab of HX, and supported by a start up fund (School of Medicine) and a faculty seed grant of Southern Illinois University Carbondale to HX.

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