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Prepulse Modulation of Auditory Startle Reflex with Different Stimulus Onset Asynchronies

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ABSTRACT

Introduction: Startle reflex is measured by delivering a sensory stimulus and measuring eyelid closure in human beings to investigate the neurophysiology of information processing. However, the characteristics of startle reflex can be modified by weaker prepulse. Since this modification is increasingly being associated with various psychiatric disorders, it is worth exploring the prepulse modification of startle reflex.

Aim: The aim of the present study was to explore the differential modulatory effect of auditory stimulus onset asynchronies on startle response magnitude as well as latency.

Materials and Methods: The study was conducted on 30 healthy right handed male subjects aged 18-40 years. Auditory prepulse was delivered binaurally which was followed by startle stimuli. The startle reflex was recorded from orbicularis oculi muscle by electromyography. Inter stimulus interval was varied in different trials from 30 ms to 1000 ms. The startle reflex from different trials were later analysed for response latency and magnitude.

Results: The results of the present study show that latency of the response was not different with different Stimulus Onset Synchronies (SOA's). However, the magnitude of the response was inhibited with SOAs 60 ms, 120 ms and 250 ms while facilitated with SOA 1000 ms when compared to baseline. SOAs 30 ms and 500 ms had no effect on the response.

Conclusion: Differential response to different prepulse to pulse inter stimulus interval implies that temporal presentation of stimuli is very important for priority coding. Very small SOA's are important for priority coding of the stimulus. However, with much larger SOA's, the first stimulus serves as an orienting response for the next stimulus.

Keywords: Electromyography, Inter stimulus interval, Pre-attentive processing, Stimulus encoding

INTRODUCTION

Startle reflex is a ubiquitous response to abrupt and intense stimulation reflecting fight or flight reaction. This reflex response is usually measured as eyelid closure in human beings after delivering a sensory stimulus [1]. The reflex can be easily recorded and quantified. Thus, it is a non invasive tool used to investigate the neurophysiology of information processing and has potential applications in psychology, psychiatry and psychopharmacology.

Although the startle reflex is not under voluntary control, it can be modified by different treatments. For e.g., if the startle inducing stimuli is preceded by a weaker auditory stimuli, startle response is inhibited [2]. This is known as Prepulse Inhibition (PPI). PPI is generally believed to be a result of automatic or pre-attentive processing of prepulse and is thought to reflect low-level gating of information processing [3]. Due to this gating, an individual can selectively divert attentional resources to salient stimuli.

The investigation of PPI has become increasingly important for multiple reasons. First, PPI helps to study the modulation of startle reflex [2]. Second, PPI provides a low-level mechanism of sensorimotor gating [3,4]. Third, several psychiatric disorders, such as schizophrenia, are associated with deficits in sensorimotor gating, in particular PPI [5,6].

Prepulses can be given at varying SOAs. The temporal limits of the time period of "gating" attributed to the prepulse are empirically determined to be approximately 60-500 inter stimulus interval [7]. However, there is paucity of literature showing effect of prepulse on startle reflex modulation using different SOA's. Since, PPI at different SOAs may reflect different stages of information processing [8]; it is worth exploring the effect of different SOA's on PPI modulation. Additionally, much of the PPI literature stresses on the magnitude of the observed startle reflex, it should be noted that there are also effects on latency of the startle response that are quite important [9]. The rapidity with which the stimulus leads to startle reflex can be measured by latency of onset and peak of startle reflex. Thus, in this study we explored the differential modulatory effect of auditory SOAs on startle response magnitude as well as latency.

MATERIALS AND METHODS

The ethical clearance for the study was obtained from the Institutional Ethics Committee. This cross-sectional study was conducted in Department of Physiology, All India Institute of Medical Science, New Delhi, India from August 2007 to July 2008. Since studies had found difference in startle response parameters in males and females [10], we recruited only male subjects to keep the sample homogeneous. Thirty healthy right handed male subjects aged 18-40 years were recruited from the institute population. All the subjects were informed about the nature of the study and consent was obtained. All subjects were asked to fill a questionnaire that included questions on their personal and family history of neurological disorders, past history of illness and medication usage. Subjects with a history of smoking or any other drug abuse, subjects suffering from diabetes or other metabolic disorders, and presence of any other chronic debilitating illness were excluded from the study. Brief clinical examination was done to rule out presence of any neurological disorders which might potentially affect the outcome of the study. Baseline hearing test was done on each subject for screening of hearing impairment. Subjects with hearing threshold above 15 dB in either ear were recruited.

Subjects reported in the Neurophysiology lab in Department of Physiology, early morning (9 AM) after taking a light breakfast.

Experiments were done in a dimly lit and soundproof room. Skin below each eye was cleaned with pads containing alcohol. Solid Ag-AgCl electrodes were placed under each eye on orbicularis oculi muscle, 10-15 mm below the pupil and about 15-20 mm below and lateral to the outer canthus of both eyes. Signal ground electrode was placed on forehead and ground electrode was placed on centre of ear lobes.

Recording was done in lying down position with eyes closed. During recording, subjects were instructed to stay awake. For baseline auditory startle reflex, a noise burst (80 dB, 50 ms) with instantaneous time rise was delivered binaurally via head phones. Real time analyser (Yoshimasha Electronic Inc.) was used to quantify the sound intensity. Bipolar Electromyograph (EMG) was recorded from orbicularis oculi muscle using 20-500 Hz bandpass and notch filter by Neuropack-8 (Nihon Kohden, Japan). Startle response EMG wave form was recorded from the averaged EMG recording obtained after 12 noise bursts delivered at random intervals of 8-25 ms and was stored for offline analysis later.

Auditory prepulse was white noise, (30 ms duration, and 40 dB amplitude) with instantaneous time rise, presented binaurally via head phones. The prepulse digitally triggered Neuropack-8 (Nihon Kohden, Japan) to deliver startle inducing noise burst (80 dB) after a pre-programmed SOA. Event blocks with varying SOAs (30, 60, 120, 250, 500 and 1000 ms) were pre-programmed using superlab software. Thus, a set of six blocks with SOAs (30, 60, 120, 250, 500 and 1000 ms) was created. Each prepulse modulation was repeated 12 times with random inter-trial interval of 8-25 second. Inter-block interval was two minutes.

The EMG wave form was rectified and scored for onset latency, peak latency, peak amplitude and area under the curve. Trials with excessive eye movements or excessive muscle artifacts were excluded from the analysis. The magnitude of PPI was calculated as PPI ratio, by subtracting prepulsed response from the non prepulsed response and dividing it with non prepulsed response multiplied by 100.

STATISTICAL ANALYSIS

All analysis was performed using SPSS 15.0. Peak amplitude and area under curve for different SOAs were normalised with pulse alone amplitude and area under the curve respectively. Normality of data was checked using Shapiro Wilk test. Levene's statistics was used to check homogeneity of variance.

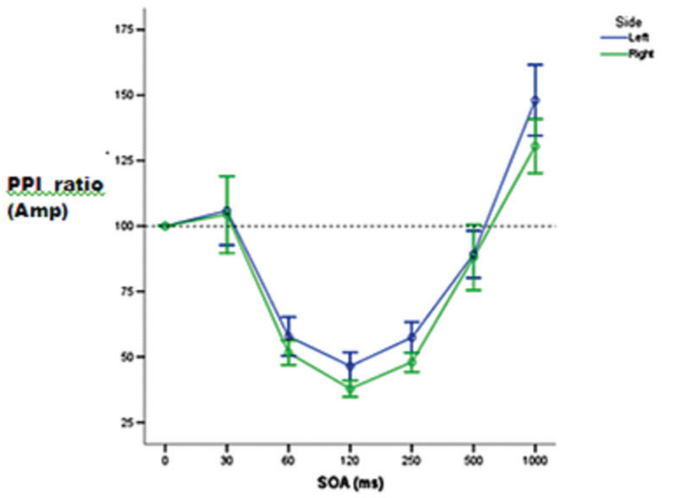
For assessing the effect of different SOAs on onset latency, peak latency, peak amplitude, area under the curve one way Analysis of Variance (ANOVA) was done for both left and right side reflexes separately. Post hoc multiple comparison was done using Tukey's test. All statistical tests were considered significant at p-value less than 0.05.

RESULTS

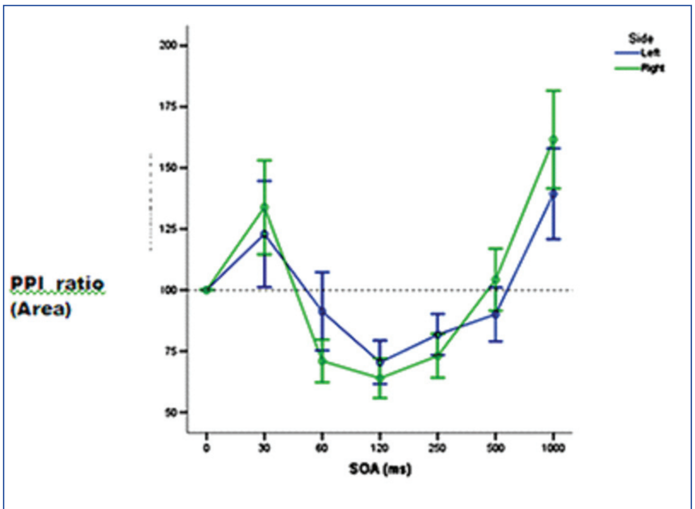
Bipolar electromyograph characteristics in terms of onset latency, peak latencies, peak amplitude and area under the curve at different SOA for auditory prepulse has been presented in [Table/Fig-1] and PPI ratio for amplitude and area under curve are shown in [Table/Fig-2,3]. The difference between right and left side for all the parameters

Stimulus onset asynchrony	Onset Latency (ms)		Peak latency (ms)		Peak amplitude (μv)		Area under the curve (μv)^2	
	Right	Left	Right	Left	Right	Left	Right	Left
Baseline	31.32±6.84	32.60±11.69	63.93±18.49	67.21±20.46	36.03±19.99	33.77±18.06	688.30±528.78	665.00±411.02
30	30.49±7.47	30.30±7.55	57.25±16.84	58.27±16.68	36.93±29.25	35.23±29.97	855.23±698.65	649.87±414.56
60	29.65±7.03	29.87±7.51	59.67±16.15	57.70±17.57	19.53±16.01	19.03±15.87	381.17±214.00	480.90±298.63
120	31.29±8.48	29.57±6.98	61.49±14.50	59.74±16.45	14.30±11.82	15.67±14.01	363.42±243.99	400.87±297.12
250	29.66±7.00	29.81±7.57	57.87±18.35	58.00±20.35	16.93±11.43	19.17±14.98	406.07±258.49	474.60±345.58
500	28.56±6.12	29.63±5.72	58.18±18.71	55.89±16.57	29.40±23.86	29.07±22.63	597.37±376.58	575.57±571.30
1000	28.92±4.65	28.94±6.49	62.04±14.29	60.56±11.57	45.87±28.83	46.43±29.87	950.80±628.54	833.30±701.17

[Table/Fig-1]: Effect of different SOAs and prepulse modality on onset latency of startle reflex (Mean±SD).



[Table/Fig-2]: Effect of different SOAs on peak amplitude of startle reflex (auditory prepulse).



[Table/Fig-3]: Effect of different SOAs on area under the curve of startle reflex (auditory prepulse).

was analysed by paired t-test which did not show any difference between the responses of right and left side. Owing to it, effect of different SOAs duration (30, 60, 120, 250, 500, 1000 ms) on prepulse inhibition characteristics in terms of onset latency, peak latencies, peak amplitude and area under the curve was analysed using ANOVA for both right and left sides using "SOA*Side" as a model.

The results from one-way ANOVA for onset latency, peak latencies, peak amplitude and area under the curve at different SOA duration has been presented in [Table/Fig-4]. The onset and peak latencies were not significantly different at different SOAs. The peak amplitude and area under the curve of the EMG recordings with different SOAs were significantly different (p<0.001). The results of post hoc multiple comparison test (Tukey's test) between different SOAs showed that peak amplitude and area under curve of EMG response was significantly less with SOAs 60 ms,120 ms and 250 ms while they were significantly more with SOA 1000 ms when compared to baseline. SOAs 30 ms and 500 ms had no effect on EMG characteristics of startle response when compared to baseline [Table/Fig-4].

No significant difference was observed at SOA 30 ms and 500 ms. At shorter SOA of 30 ms, the prepulse may have been sensed but not processed thus leaving the attentional resources free to encode and process other stronger stimuli. This may be due to the fact that 30 ms duration between prepulse and pulse is too small to allow the encoding and processing of weak stimuli thus not leading to attenuation of subsequently coming stimuli (startle inducing stimuli) as the attentional resources do not have enough time to get engaged in processing prepulse. This also implies that when two stimuli are presented successively in a very short interval (30 ms), attentional resources are allocated to the stronger stimuli in preference to weaker stimuli. With smaller inter stimulus interval, both stimuli are processed in different streams parallely [16]. Thus, at these SOA louder stimuli automatically get high priority code. This may give a survival benefit in the sense that CNS perceives louder, life threatening stimuli in preference to other stimuli and produces an appropriate response automatically. At the pre-attentive level, the preference is given to crude features of stimuli like intensity, pitch, amplitude and the brain automatically responds to louder, brighter stimuli. It seems crude features are more important pre-attentively at shorter lead intervals [17].

Variables	Auditory prepulse		Post hoc analysis for different SOAs [baseline (a), 30 (b), 60 (c), 120 (d), 250 (e), 500 (f)]					
	F	p-value	30	60	120	250	500	1000
Onset Latency (ms)	0.38	0.977						
Peak Latency (ms)	1.02	0.428						
PPI Ratio (Amplitude)	14.76	0.000*		a,b	a,b	a	c,d,e	a, b,c,d,e,f
PPI Ratio (Area)	4.87	0.000*		b	b			a, c,d,e,f

[Table/Fig-4]: One-way analysis of variance (ANOVA) test and post hoc analysis for auditory, prepulses for baseline and different SOAs at 30, 60, 120, 250, 500, 1000 ms for right and left sides (Model=SOA*Side).
Alphabets (a,b,c,d,e,f) represent statistically significant differences in a row with respective SOA (p<0.001)

DISCUSSION

The aim of the present study was to study the effect of sensory prepulses on auditory startle response with different SOAs. The noise bursts used to evoke startle were safe in terms of potential effects on the auditory system because of very short duration. The 50 ms/80 dB sounds are well below the recommended limits stated in the documentation of various safety regulations. The Occupational Safety and Health Administration (OSHA), USA recommends that exposure to impulsive or impact noise should not exceed 140 dB peak SPL [11]. The National Institute on Deafness and other Communication Disorders (NIDCDs), USA advises no more than 15 minutes unprotected exposure at 100 dB sounds [12].

The results of the present study show that latency of the response was not different with different SOA's. However, the magnitude of the response was inhibited with SOAs 60 ms, 120 ms and 250 ms while facilitated with SOA 1000 ms when compared to baseline. SOAs 30 ms and 500 ms had no effect on the response.

The prepulse inhibition of startle reflex with SOAs 60 ms, 120 ms and 250 ms observed in present study is consistent with previous studies which observed strongest inhibition of startle with a prepulse startle Inter Stimulus Intervals (ISIs) between 40 and 250 ms [2,13]. This is in accordance with the "protection of processing hypothesis" proposed by Graham FK [7,14] which is also recognised as a model of sensorimotor gating [3]. It suggests, encoding and analysis of first stimulus attenuates all subsequent stimuli until it's encoding is completed. It reduces the available capacity of the attentional system [15] during the processing window and subsequent startle stimulus is perceived less intense. Such suppression also means that at these SOAs, stimuli are not given preferences according to their crude features. In fact the weak prepulse is protected against any other distracting stimuli so that it can be processed completely for finer features also.

Higher SOA of 500 ms is enough to completely process a stimulus [18] and irrelevance of it is perceived. As a result of this that stimuli is filtered out of attentional resources or it decays itself. The attentional resources are thus free to process another stimulus, resulting in no suppression of startle response. Our findings also show PPI enhancement of startle response at high inter-stimulus intervals of 1000 ms. Facilitation at 1000 ms can be explained by the generalised orienting response caused by the prepulse after its processing is over [19]. This leads to greater allocation of attention to stronger stimuli. Expectancy may also play a role in this reflex facilitation at 1000 ms [20].

Responses to various sensory stimuli are attenuated pre-attentively depending on the priority code in this stimulus-laden world. Differential response to different prepulse to pulse inter stimulus interval implies that temporal presentation of stimuli is very important for priority coding. Thus, in the presence of several stimuli, the one with the precedence wins [15]. For very small SOA's, crude feature of the stimuli become important for priority selection. Once the stimulus enters into encoding and perceptual analysis, it becomes relevant over other stimuli presented in that window and even weaker stimuli is processed preferably and protected against any sort of distraction from other stimuli.

Since, factors that affect PPI may affect right and left startle reflex unequally, laterality issues in PPI measures have been considered important in normal individuals. As some evidence had supported a normal lateralisation in blink reflex magnitude (right>left) [21], we recorded the response from both sides. Our results did not show any significant difference when we compared the EMG response of right and left sides. This is in contrast to studies which found consistent larger area under the curve of EMG response on right side than on the left [22]. However, laterality issues may not be significant with binaural stimulation [23]. Thus, it is evident that unilateral orbicularis oculi EMG can subserve the purpose in automatic attention studies using startle reflex as a paradigm if binaural stimulation is used.

LIMITATION

To consider the implications of the present study, some limitations need to be warranted. Firstly, we included only the healthy subjects in this study, therefore the results may not be extended to whole spectrum of diseases like obsessive compulsive disorders, attention deficit disorders etc. Secondly, we have used same modality prepulse only. Thus, the findings cannot be generalised to the differential modulatory effect of other modality prepulses on startle response. Thus, for future studies we recommend to compare the effect of different sensory modalities to interpret the results in environmental context.

CONCLUSION

Differential response to different prepulse to pulse inter stimulus interval implies that temporal presentation of stimuli is very important for priority coding. For very small SOA's, crude feature of the stimuli becomes important for priority selection. Once the stimulus enters into encoding and perceptual analysis, it becomes relevant over other stimuli presented in that window and even weaker stimuli is processed preferably and protected against any sort of distraction from other stimuli. However, with much larger interval, the first stimulus serves as an orienting response for the next stimulus. This study will help to better understand the phenomenon of attention, pre-attentive and post-attentive processing of stimuli. Since, startle reflex is increasingly being used to study psychological and psychiatric disorders, this study will also help to explore the pathophysiology of disorders ranging from anxiety to schizophrenia where attention deficits seems to play an important role.

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