

LEAD-STIMULATION EFFECTS ON HUMAN STARTLE EYEBLINK RECORDED BY AN ELECTRODE HOOKUP¹

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The purpose of this study was to investigate the utility of the electrode hookup technique in studying the startle eyeblink reflex and the lead-stimulation effects upon it. In Experiment I, 60 subjects were tested to identify the distributional properties of the reflex amplitude. The results showed that the amplitude was distributed normally when a log-transformation was performed. Positive correlations between the measures of reflex and spontaneous blink suggested that individual factors, e.g., the size of eye and responsivity, must be controlled in the design of the experiment. In Experiment II, nine subjects were tested to reduplicate the experiment of Graham, Putnam, and Leavitt (1975) using the electrode hookup recording technique. Both results were similar to each other in spite of the difference in measuring technique. The validity of the present technique was thus established.

The amplitude of the acoustic startle reflex is modified by a relatively weak stimulus presented prior to a reflex-eliciting stimulus. When the interstimulus interval (ISI) between the lead stimulus and reflex-eliciting stimulus is shorter than a few hundreds msec, the amplitude of the reflex is inhibited. On the other hand, when the ISI is prolonged beyond 400 msec or more, the startle reflex is facilitated (Yamada & Miyata, 1979).

These effects are known as "lead-stimulation effects" and have been observed in various species, e.g., pigeons (Stitt, Hoffman, Marsh, & Schwartz, 1976), rats (Hoffman & Wible, 1969; Ison

& Hammond, 1971), rabbits (Ison & Leonard, 1971) and humans (Graham et al., 1975; Krauter, Leonard, & Ison, 1973).

According to previous reports, these lead-stimulation effects seem not to be simply the product of peripheral phenomena, i.e., receptor fatigue or response interference (e.g., Ison & Hammond, 1971). Since these effects are caused through central information processing mechanisms (Braff, Stone, Callaway, Geyer, Glick, & Bali, 1978; Graham, 1975), the experimental study with human subjects has a special significance.

In human studies, the acoustic eyeblink reflex to the loud stimulus is regarded as the startle reflex, and it has usually been recorded by the standard fine-wire hookup. This hookup has the following disadvantages in recording eyelid movement. First, a physical stress on the lid produced by the attachment of hookup may disturb the "real movement" of the lid. Second, as subjects become aware of what responses are measured, they may respond voluntarily or threateningly to the stimulus. Lastly, the attachment may pro-

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duce an unpleasant feeling in the subjects. Though how these factors influence the startle reflexes are unknown, we would like to keep them from confounding the target effects of "lead-stimulation" as much as possible.

Osborne, Roach, Gendreau, and Gendreau (1974) used an electrode hookup and succeeded in bypassing complicated individual reactions to the standard mechanical method. This new recording technique is fundamentally the same as the vertical electrooculography (EOG). Throughout the present two experiments the eyeblink was measured by this method recommended by Osborne et al. However, since the reliability of the amplitude of the eyeblink reflex measured by this method has not been established, in Experiment I the distributional properties of the reflex amplitude and the correlations with other measures were examined first. After that, we tried in Experiment II to replicate the experiment of lead-stimulation effects performed by Graham et al. (1975).

EXPERIMENT I

Purpose

In this experiment, we attempted to clarify the distributional properties of the eyeblink reflex amplitude measured by the electrode hookup method. It is important to identify the distribution of the target measure for the purpose of statistical analyses. It is known that the amplitude of electrophysiological responses, e.g., GSR, and the psychological sensory responses distribute normally when they are transformed into a logarithmic scale. Graham and Murray (1977) and Yamada and Miyata (1979) employed logarithmic transformation in the analyses of eyeblink amplitude with the mechanical and photo-sensor technique, respectively. The evidence for the validity of this transformation in analyzing the amplitude of the eyeblink reflex, using the electrode hook-

up technique, will be provided in the present experiment. Additionally, we will propose some topics that are important in making a valid experimental design from the viewpoint of correlational analyses.

Method

Subjects. Sixty undergraduates, 31 females and 29 males, two junior-high and two graduate students served as subjects. Mean age was 21.0 with a range of 14-30 yrs.

Apparatus and procedure. The testing room was an air-conditioned, sound-attenuating chamber of 215×135×177 cm. The subject, seated in a comfortable chair, received startle stimuli binaurally through Pioneer SE-205 headphones. The startle stimulus was a 50-msec burst of white noise, which was recorded on a tape recorder and fed into the audio-amplifier. The stimulus was gated on and off with rise and fall times less than 1 msec, controlled by National reed relay NR-H-5V. The intensity level was calibrated on the C scale of the Rion NA-07 sound level meter at the headphones. Monaural intensity of the noise was 110 dB. Stimulus presentation and timing were controlled by a 6-channel digital preset timer made of IC circuits and a paper tape reader.

Vertical EOG was picked up from the left side of the subjects' face with Nihon Koden Sintered Ag-AgCl miniature skin electrodes, NT-212U. The positive electrode was placed just above the eyebrow; the negative electrode was placed just below the cheekbone, beside the nose. A ground electrode was placed on the fleshy part of the cheek (after Osborne et al., 1974). Attachment of the electrode was made with Nihon Koden electrode paste and collars. The electrodes were coupled to a San'ei Bio DC-amplifier Model 1117 and recorded by a San'ei pen-galvanometer (Rectigraph 8S) with a paper speed of 50 mm/sec. The gain was arranged to give 40 mm of trace deflection for 1 mV.

Electrocardiogram (ECG) was also recorded from the Lead-I electrode placement. Electromyogram (EMG) from the region of orbic-

ularis oculi ($TC=.01$) was also monitored concomitantly with EOG and ECG, but it is not reported in this article.

Subjects were instructed to remain awake and fixate a green LED, which was 1 m ahead the subjects. Each subject rested about 5 min in the dark room. After this rest period, the first startle stimulus was presented. About 1 min later, another startle stimulus was presented.

Analysis of responses. The eyeblink reflex to the startle stimulus was identified with the latency criterion, in which only the reflexes beginning in a window 20–120 msec after startle stimulus onset were picked up. The peak amplitude was measured in μV and then transformed into a logarithmic scale. If the lid was partially closed at the time of the startle stimulus onset, the trial was excluded. The onset and peak latencies were also measured on a msec scale. Spontaneous eyeblinks and heart rate (HR) were counted during the rest period, intertrial interval (ITI), and post trials for the purpose of correlational analyses.

Results

Distribution of eyeblink amplitude. The number of obtained samples was 57 in the first trial. The mean raw (μV) and transformed (log unit) scores were 303.4 ($SD=212.0$) and 2.37 ($SD=.375$), respectively. Hypothesizing that the amplitude score would distribute normally in the population, a theoretical distribution curve was calculated by using the sample mean and standard deviation. It is illustrated with a sample distribution curve in Fig. 1. As shown in Fig. 1, skewness is positive in the case of the raw score and negative in the transformed one. In order to estimate the fitness of the sample distribution with the theoretical normal distribution, the chi-square test was performed for the two scores. The sample distribution of the raw score differed significantly from the theoretical one ($\chi^2=11.83$, $df=4$, $p<.01$), but not in the case of transformed one ($\chi^2=4.55$, $df=3$, $.2 < p < .3$).

In the second trial, the number of samples was 55, the mean raw and transformed scores were 326.4 ($SD=209.7$) and 2.42 ($SD=.302$), respectively. The results of chi-square tests proved that both scores fit the theoretical normal distributions (a raw score, $\chi^2=8.10$, $df=4$, $.05 < p < .10$; a transformed score, $\chi^2=6.33$, $df=3$, $.05 < p < .10$).

From these results, it is clear that the logarithmically transformed scores fit the theoretical distribution better than the raw scores.

Latency measure. The mean onset latencies, the time from the stimulus onset to the rise of the reflex, were 61.5 msec ($SD=12.8$) and 55.8 msec ($SD=13.3$), in the first and second trial. The onset latency was shortened significantly from the first to the second trial ($t(52)=2.89$, $p<.01$). The mean peak latencies, the time from the rise to the peak of the reflex, were 68.4 msec ($SD=23.4$) and 72.1 msec ($SD=18.8$), in the first and second trial. The peak latency was lengthened a bit

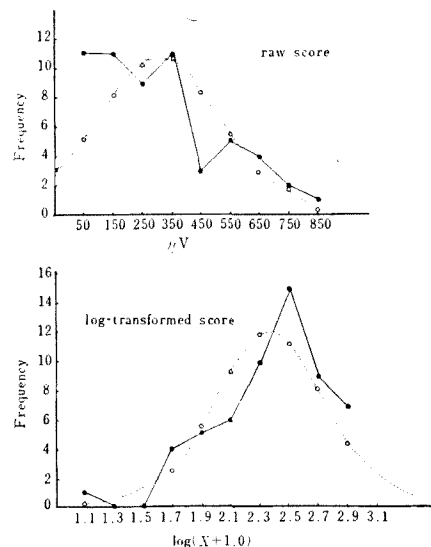


FIG. 1. Frequency distributions of raw (top) and transformed (bottom) eyeblink amplitude scores with a theoretical normal distribution (dashed line) for the first trial samples from 57 subjects.

TABLE 1
Pearson correlation scores between amplitude
and latency measures in each trial

Latency measure	Amplitude measure		
	Trial	1st	2nd
Onset latency	1st	.001 (57)	-.031 (52)
	2nd	-.069 (52)	.367* (55)
Peak latency	1st	.567* (57)	-.186* (52)
	2nd	.392* (52)	.562* (55)

Note. (): Number of samples. * $p < .01$

from the first to the second trial, but the increase was not significant but close ($t(52)=1.70$, $p < .10$). To estimate the correlation between latency and amplitude measures, Pearson's correlation scores were calculated and summarized in Table 1. As shown in Table 1, the amplitude correlated more highly with the peak latencies than with the onset latencies.

Spontaneous blink activity. The mean number of spontaneous blinks during the rest period was 26.0 per minute ($SD=15.8$). The mean amplitude and peak latency of three spontaneous blinks prior to the first trial were 2.56 in log units ($SD=.205$) and 94.7 msec ($SD=19.0$). The correlations between these three spontaneous blink and reflex measures were summarized for each trial in Table 2. As shown in Table 2, the reflex amplitude, but not the latency measures, correlated

positively with spontaneous blink frequencies. For both amplitude and peak latency, there were positive correlations between spontaneous and reflex blinks.

Other measures. Mean HR during the rest period was 77.0 bpm ($SD=11.4$). The correlations between HR and the various blink measures were all near zero (e.g., HR vs. reflex amplitude, $r=.089$ and $-.064$ in the 1st and 2nd trial). The sex difference didn't affect any eyeblink measures except for one case. In males, the frequency decreased about three per minute, but in females, increased about five per minute from the rest period to the post stimulus period. This difference between sexes was significant ($t(57)=2.79$, $p < .01$).

Discussion

From Experiment I, the following two points were suggested. First, the amplitude of the startle eyeblink reflex, measured with the electrode hookup method, showed the normal distribution in log units, but not in raw scores. Transformation into the log scale was thus valid and should be performed, especially in the experiment including some conditions in which inhibition of the startle reflex is expected. Because inhibition below zero can not be expected to occur, distribution will skew in the inhibitory condition, and therefore means and standard deviations

TABLE 2
Pearson correlation scores between reflex measures and spontaneous blink measures

Spontaneous blink	Trial	Reflex measures		
		Amplitude	Onset latency	Peak latency
Number of blinks	1st	.295* (57)	.083 (57)	-.029 (57)
	2nd	.309* (55)	.165 (55)	-.058 (55)
Amplitude	1st	.359* (57)	-.042 (57)	.168 (57)
	2nd	.466* (55)	-.178* (55)	.156 (55)
Peak latency	1st	.049 (57)	.080 (57)	.241* (57)
	2nd	.133 (55)	-.068 (55)	.371* (55)

Note. (): Number of samples. * $p < .01$

will be proportional. Analyses must be therefore carried out on log-transformed measures ($\log(x+1.0)$).

Second, the positive correlations between spontaneous blink activities and reflex amplitude suggested that the size of the eye and the responsivity of the blink may influence the amplitude of the reflex blink. A large experimental error will be produced if these factors are ignored. In order to discriminate the treatment effect from these error factors, a within-subjects design must be used. In a between-subjects design, it is necessary to match both the frequency and amplitude of the spontaneous blink across groups.

EXPERIMENT II

Purpose

The purpose of this experiment is to replicate Graham et al. (1975) with the electrode hookup method. The effects of the ISI and the type of lead stimulus upon the amplitude of the startle eyeblink reflex were tested in a within-subjects design.

Method

Subjects. Nine undergraduates, four females and five males, served as subjects. Their mean age was 22.0 with a range of 18–26 yrs.

Apparatus and procedure. The lead stimulus was a 1,000-Hz pure tone, generated by a Torio audio-generator AG-202A and its intensity was 70 dB at the headphones. The startle stimulus and almost all of the other apparatus were the same as in Experiment I.

Each subject received 81 trials arranged in a 9 by 9 Latin square with nine types of trials ordered in nine blocks of trials, so that each type occurred once in the block and at a different place within each block. In each block there was one control trial in which the startle stimulus was presented alone and eight lead-stimulation trials. These lead-stimulation trials were taken from the 2 by 4 factorial design with two types of lead stimulus (continuous- S_1 and discrete- S_1) and four levels of ISI (200, 800, 1,400, and 2,000 msec). Con-

tinuous- S_1 continued until the onset of the startle stimulus, but the discrete- S_1 continued for only 50 msec. The same Latin square was used for all nine subjects, but each subject began the session at a different row of the square and proceeded through subsequent rows in sequence. The order of presentation was thus balanced across subjects. The interval between trials varied randomly between 25 and 45 sec with a mean of 35 sec.

Analysis of responses. The amplitude of the eyeblink reflex in log units was analyzed as in Experiment I. An analysis of variance, three-way classification with nine observations in cells was employed to estimate experimental factors ($ISI \times S_1\text{-type} \times \text{Trials}$). In the analysis of this experiment, data for discarded trials were estimated by interpolation between adjacent trials and for non-response trials by assigning an amplitude score of 0. Discarded trials were 3.0% of total trials, and their number did not differ among the treatment conditions.

Results

The mean amplitude of the eyeblink reflex for each S_1 -type condition is illustrated in Fig. 2 as a function of the ISI. As shown in Fig. 2, the amplitude increased as the ISI was lengthened in both S_1 -type conditions but was larger in the continuous- S_1 condition than in the discrete- S_1 condition. Results of an ANOVA indicated that the main effects of the ISI

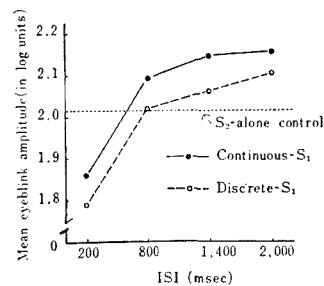


FIG. 2. Mean eyeblink reflex amplitude as a function of interstimulus interval (ISI) between a 70-dB, 1,000-Hz lead stimulus and a 110-dB, white noise.

($F(3, 24)=18.72$, $p<.005$), S_1 -type ($F(1, 8)=15.19$, $p<.001$), and Trial ($F(8, 64)=6.32$, $p<.005$) and the three-factor interaction ($F(24, 192)=1.75$, $p<.05$) were significant. In order to interpret the three-factor interaction, amplitude scores were illustrated in Fig. 3 for all conditions as a function of trials. Showing in Fig. 3, the habituation was shown in all conditions except for in discrete- S_1 in the 200-msec ISI. The amplitude in this condition was the smallest throughout early trials.

To examine the modification effect, the amplitude score for each lead-stimulus condition was separately compared with a S_2 -alone control condition, using two-tailed t tests. The inhibitory effect at the 200-msec ISI and the facilitatory one at the 2,000-msec ISI were significant with both discrete and continuous S_1 condi-

tions (C-200, $t=2.66$; D-200, $t=3.83$; C-2,000, $t=3.45$; D-2,000, $t=3.23$). The facilitatory effect at the 1,400-msec ISI was also significant only in the continuous- S_1 ($t=2.68$). In all cases, the degrees of freedom are eight and the term significant refers to $p<.05$.

The onset latency of the blink reflex was also affected by a lead stimulus, as shown in Fig. 4. The result of the ANOVA indicated that only the main effects of the ISI and S_1 -type were significant ($F(3, 24)=12.54$, $p<.001$; $F(1, 8)=5.94$, $p<.05$). The onset latency was shorter in lead-stimulus conditions than in a S_2 -alone control condition. The significant facilitation was shown at the 800, 1,400, and 2,000-msec ISIs, but not at the 200-msec ISI (C-800, $t=4.33$; D-800, $t=2.79$; C-1,400, $t=5.16$; D-1,400, $t=2.38$; C-2,000, $t=6.87$; D-2,000, $t=4.96$).

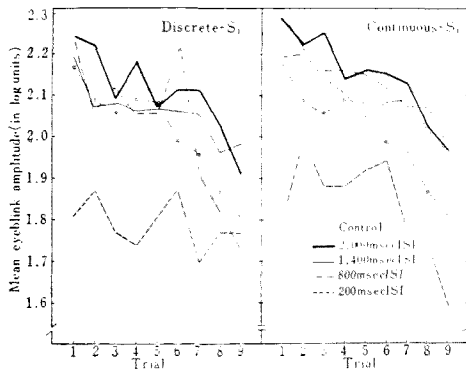


FIG. 3. Changes in mean eyeblink reflex amplitude as a function of trial for each condition.

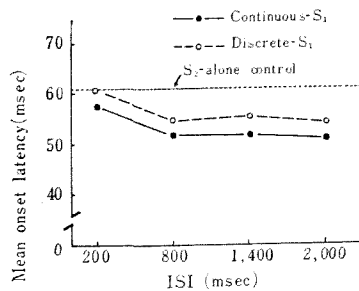


FIG. 4. Mean onset latency of the eyeblink reflex as a function of ISI.

Discussion

The results of this experiment were mainly the same as that of Graham et al. (1975), but differ in two points. First, the effects of S_1 -type did not interact with the ISI in our result. Secondly, the onset latency of the blink reflex was facilitated in each condition, as in our previous report (Yamada & Miyata, 1979) and that of Graham and Murray (1977). These two differences may come about from the method used to measure the lid movement. In our experiment, any small movements of the lid could be detected, because there was no physical stress directly on the lid. This is based on the observation that there were only four zero-responses in our data at 200-msec ISI. Graham et al. (1975, p. 164) reported that in 16% of their trials (estimated about 26 trials) the blink was completely suppressed.

In spite of the above two differences, our results were very similar to previous reports using other measuring techniques (Graham et al., 1975; Yamada & Miyata,

1979). The eyeblink reflex to the startle stimulus was inhibited in short ISIs and facilitated in long ISIs. These effects occurred in the first trial and continued until the end of the experiment, though the basal reflex amplitude decreased as a function of trials. Moreover, these effects of lead-stimulation were neither acquired nor resulting from a conditioning effect.

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