THE EFFECTS OF STARTLE STIMULUS PROBABILITY ON THE HUMAN ELECTROMYOGRAPHIC STARTLE RESPONSE

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Abstract

The amplitude and latency of the rat startle response to an intense acoustic stimulus has been significantly inhibited or facilitated by antecedent changes in sensory stimulation. Onset, terminations or stimulus changes preceding the startle stimulus by 15 to 400 ms inhibit the whole body startle response (Hoffman and Searle, 1968). The effect can be elicited using either weak brief or continuous tones. Inhibition also appears cross-modally using visual lead stimuli and acoustic startle (Ison and Hammond, 1971). Beyond 400 ms the inhibition dissipates to control levels, however, continuous prestimulation at lead intervals beyond 2000 ms facilitates both amplitude and latency of the response.

Graham, Putnam and Leavitt (1975) have replicated the short lead interval inhibition in humans using the blink reflex to an acoustic stimulus as a measure of startle. Similarly, Brown (1975) has found continuous prestimulus facilitation employing only a 2000 ms lead interval. However, uncertainty created by within-subject variation of lead stimulus duration and interval length in the Graham et al. study (1975) produced significant startle 2000 ms after a brief lead stimulus, an effect not present in animal startle research. Discrete lead stimulus facilitation was not present when lead stimulus duration and interval were held constant (Bloch, 1972). Graham (1975) has proposed that discrete lead stimulus facilitation is induced by the termination

of orienting-attentional processes elicited by lead stimulus onset and representing the subject's efforts to reduce uncertainty as to startle events.

The present study was designed to further identify the factors necessary for discrete lead stimulus startle facilitation and to investigate the orienting and attentional/arousal processes participating in the modulation of the EMG startle response. Subjective set was manipulated by instructions to the subject informing him that onset of one of three discrete lead stimuli would signal a certain or an uncertain startle stimulus exactly two seconds later. Lead stimulus duration and interval length were thus held constant in the present study. It was predicted that the low startle certainty condition would elicit startle amplitude and latency facilitation, while the high certainty condition would not. Cardiac deceleration and CNV amplitudes were also expected to be greatest in the uncertainty condition, reflecting the orienting and attentional/arousal processes whose offset was suggested to mediate the facilitory effect. As expected, significant EMG startle amplitude and latency facilitation was elicited in the low certainty condition. Startle latency facilitation was also elicited in the high certainty condition, contrary to expectation. Significant heart rate deceleration and CNV amplitudes were present in both the high and low startle certainty conditions but did not differentiate startle certainty nor correlated with startle facilitation. Shape of the CNV, however, did differentiate the two levels of startle certainty. The low startle certainty condition elicited a curved shape CNV response similar to a "type A" CNV, previously associated

with interval uncertainty (Tecce, 1972), while the high certainty condition showed a monotonic rise through the lead interval. These results suggest that CNV is differentially sensitive to arousal elicited by either lead stimulus or terminal startle event variability. A physiological mechanism modulating reflex amplitude and latency, which is also sensitive to levels of cortical arousal was discussed.

THE EFFECTS OF STARTLE STIMULUS PROBABILITY ON THE HUMAN ELECTROMYOGRAPHIC STARTLE RESPONSE

Introduction

In mammals the startle response to an intense acoustic stimulus is a rapid sequence of flexor contractions that begins in the face and neck and spreads down the body resulting in a crouching position (Landis and Hunt, 1939). The amplitude and latency of the rat startle response can be significantly inhibited or facilitated by changes in the antecedent visual or auditory background.

Short Lead Interval Effects

Onset of a weak, discrete or continuous, lead stimulus 15 to approximately 400 ms before startle stimulus onset inhibits the startle reflex (Hoffman and Searle, 1968; Hoffman and Wible, 1969). Beyond 400 ms the inhibitory effect of a discrete lead stimulus dissipates to control levels, or more specifically, to the level of those trials on which only a startle stimulus was presented. The inhibitory effect at short lead intervals does not depend upon classical conditioning or learning since the effect appears in the first pairing of the lead and startle stimuli (Hoffman and Wible, 1970; Ison and Leonard, 1971). The protective middle ear reflex has also been eliminated as a possible mediating mechanism of the inhibitory effect because lead stimuli are too weak to elicit the reflex. Through classical conditioning a weak stimulus could elicit the reflex, however, maximum startle inhibition occurs at intervals too short for optimal conditioning in

addition to the evidence of inhibition occurring on the first trial.

In his early research, Hoffman proposed that a brainstem startle center mediated the short lead interval inhibitory effect (Hoffman and Searle, 1968). After an initial 10 ms excitatory interval, during which latency is facilitated, the center becomes refractory to further stimulation. By 2000 ms the center recovers to pre-stimulation levels. More recent work of Hoffman and his collaborators has demonstrated that termination or a change in the frequency of a continuous lead stimulus can also inhibit the startle response at short lead intervals (Stitt, Hoffman and Marsh, 1973; Stitt, Hoffman, Marsh and Boskoff, 1974). Because startle is sensitive to stimulus change per se, Stitt et al. (1974) proposed that a process related to the orienting response activates the startle center and produces the inhibitory effect.

Long Lead Effects

Presentation of a brief lead stimulus more than two seconds before a startle stimulus does not modify the startle response. However, continuous lead stimulation beyond 2000 ms facilitates both the amplitude and latency of the startle response in rats (Hoffman and Searle, 1965). Stitt et al. (1974) suggested that acoustic input at the onset of the startle stimulus affects the mechanism responsible for startle facilitation. Ison and Hammond (1971), however, have proposed a common mechanism to account for both the short lead inhibitory and the continuous lead stimulus facilitory effects involving activation of the forebrain system. More specifically, they have suggested that the orbital surface of the frontal lobe and its

projections to specific inhibitory and facilitory sites in the reticular formation mediate the startle response. In addition, Ison, Hammond and Krauter (1973) have proposed that this system is particularly sensitive to levels of generalized cortical arousal.

Human Startle

Graham, Putnam, and Leavitt (1975) have successfully replicated the short lead interval inhibitory effect in humans using the blink response to an intense acoustic stimulus. At lead intervals of 30 and 60 ms, however, latency of the response was facilitated,which is beyond the range of short lead interval latency facilitation found in the rat startle response (in Graham, 1975). By 200 ms inhibition of response latency is present in humans (Bloch, 1972). These short lead interval effects are the same for both discrete and continuous lead stimulation. Graham (1975) has proposed a mechanism similar to that suggested by Stitt et al. (1974) involving the orienting reflex arc. More specifically, she has concluded that a change in the antecedent background stimulation, which occurs in less than 20 ms, is responsible for the activation of the inhibitory mechanism.

At longer lead intervals of 2000 ms, both Bloch (1972) and Graham et al. (1975) found continuous lead stimulus amplitude and latency facilitation of the blink response. Graham (1975) has attributed the effect to an intensity-dependent phasic activation, which is similar to the mechanism proposed by Ison (Ison and Hammond, 1971; Ison, Hammond and Krauter, 1973).

Long Lead Interval Facilitation

Discrete lead stimulus effects at long lead intervals vary as

a function of the experimental design in which the trial is embedded. In a study using only two startle conditions, a control startle stimulus and a paired lead-startle stimulus condition, no significant differences in startle amplitude were found (Bloch, 1972). However, when the number of within-subject conditions was increased by varying the length of the lead interval and lead stimulus duration or startle stimulus intensity (Bloch, 1972; Graham et al., 1975), both significant amplitude and latency facilitation were elicited in the 2000 ms lead interval condition. The "hard-wired" mechanisms proposed by Hoffman and Ison are strictly dependent upon lead interval and sensory input preceding startle stimulus onset. These models, as proposed, cannot account for the variable presence and absence of discrete lead stimulus facilitation as the complexity of the design changes. Graham has therefore proposed that in complex within-subject designs humans engage in high-level processing during the lead interval, which leads to startle facilitation. This high-level activity is indexed by the orienting-attentional processes of cardiac deceleration and the contingent negative variation of the EEG.

Previous research (Graham, 1973) has shown cardiac slowing of one to two beats per minute elicited by simple non-signal stimuli, while larger decelerations to signal stimuli usually only occur when a behavioral response is being organized (Higgins, 1971). It was therefore unexpected that in complex startle designs both Bloch (1972) and Graham et al. (1975) found heart rate decelerations as great as three and four beats following discrete lead stimulus onset. Conversely,in a simpler design using only a single long lead interval

Brown (1975) found neither significant cardiac deceleration nor startle facilitation.

CNV, a negative shift in the EEG resting potential, has been associated with attention and cortical arousal (Tecce, 1972). Brown (1975) has found CNV to accompany startle facilitation in a continuous lead stimulus paradigm using a constant 2000 ms lead interval. Both results were attributed to an intensity-dependent mechanism activated by the continuous lead stimulus. However, when a brief lead stimulus preceded a startle stimulus by the same fixed interval both CNV and startle facilitation were absent indicating the inadequacy of the weak lead stimulus to activate the mechanism to a noticeable degree (Bloch, 1972). In complex designs CNV accompanied both cardiac deceleration and discrete lead stimulus facilitation (Bloch, 1972).

From the combined results of the above studies Graham (1975) has proposed that orienting-attentional processes elicited by the lead stimulus are essential components of discrete long lead interval startle facilitation in humans. She has further suggested that termination of these processes is directly responsible for the facilitory effects since Obrist, Webb and Sutterer (1969) have shown that irrelevant motor activity is actually inhibited during orienting.

In both the Bloch (1972) and Graham et al. (1975) studies the necessary conditions for discrete startle facilitation were shown to be contextual in nature and to depend upon variable lead intervals preceding uncertain startle events. Upon presentation of the lead stimulus the individual engages in processes aimed at the reduction of uncertainty initiated by the lead stimulus. In the constant lead

interval paradigm the brief lead stimulus contains all the necessary startle information, which minimizes uncertainty and concomitant orienting-attentional processes in the interval.

Purpose

The present study is directed to the investigation of possible startle facilitation and the accompanying orienting-attentional processes elicited by a brief lead stimulus appearing a constant 2000 ms before an intense startle stimulus. Subjective set and uncertainty in the interval will be manipulated by varying the probability of a startle stimulus occurrence after the lead stimulus. Three identical but distinguishable lead stimuli will separately signal 100%, 50%, or 0% startle stimulus probabilities. Each subject will be informed of the contingencies at the beginning of the experimental session.

If terminal event uncertainty is adequate to elicit amplitude and latency facilitation, then these effects should be present in the 50% startle certainty condition but absent in the 100% certainty condition.

In the standard CNV S_1-S_2 paired stimulus paradigm a motor response is required to the imperative stimulus (S_2). Diluting the probability of S_2 occurrence from 100% to 50% reduces the amplitude of the CNV response (Walter, 1968). Hillyard and Galambos (1967), however, found that the same reduction in S_2 probability did not reduce CNV amplitude. Thus, the effects of S_2 probability on CNV are contradictory. If, as in previous startle research, CNV accompanies uncertainty in the interval then it should appear in the 50% certainty condition but not in the 100% startle condition.

Similarly, cardiac deceleration can be expected to accompany lead stimulus elicited uncertainty in the interval. No significant cardiac response is predicted in the 100% startle condition since all necessary startle information is contained in the lead stimulus.

Method

Subjects

Subjects were 17 male undergraduate volunteers from the College of William and Mary. All participating subjects were paid two dollars per hour and informed they could leave the experimental session at any time. In response to questioning, no subject reported hearing problems, head injuries, heart disorders, or prescription medication or drugs taken in the preceding 24 hours. One subject was replaced due to a poor signal-to-noise ratio of the startle response recording, and a second subject was replaced as a result of a base level heart rate of about 130 beats per minute. Results are therefore based on 15 subjects.

Apparatus and Procedure

Stimuli. Visual lead stimuli were presented using a 9.5 cm x 16.0 cm x 5.5 cm black plastic box containing three red light emitting diodes (LED's) mounted on the side of the box facing the subject. The three LED's were 2.5 cm apart from each other horizontally across the face of the box making them distinguishable to the subject by their relative positions left, center and right. The height of the box was adjusted to a comfortable position at about eye level one meter in front of the subject's face. Each subject was seated in a soft armchair for the experimental session. Duration of each visual lead stimulus was 20 ms with instantaneous rise and fall times. Background illumination in the

laboratory was provided by fluorescent lighting. All subjects reported no difficulty in distinguishing one lead stimulus from another. All testing was performed from 6 P.M. to 11 P.M. thus maintaining constant illumination during each session.

The acoustic startle stimulus was a 50 ms, 1000 Hz square wave tone with instantaneous rise-fall time. The tone was generated by a shop-built two channel oscillator and calibrated at 0.8 volts, 50% duty cycle using a Tektronix 5103 storage oscilloscope. A pair of Grayson-Stadler TDH-39 headphones delivered the tone binaurally to the subject. The intensity of the startle stimulus was calibrated monaurally at 115 db (re 0.0002 microbars) using a General Radio 1565-B audiometer and a P-83 earphone coupler. The binaural intensity was also estimated to be 115 db.[1]. MX41/AR cushions were used to calibrate the tone. However, 001A cushions were employed during the experimental session.

Onsets, durations, and terminations of all visual and acoustic stimuli as well as the interval between the visual lead stimulus and the acoustic startle stimulus were controlled by a Monroe 1860 Programmable Calculator. The calculator also placed a +5 volt signal pulse on one channel of a Vetter C-4 FM tape recorder whenever a stimulus was presented. An additional signal pulse was placed on the same tape channel 2000 ms before any visual lead stimulus and 2000 and 4000 ms before any startle stimulus. These latter signals were recorded to obtain pre-stimulation levels of heart rate and EEG.

Stimulus Conditions. Each subject completed a 5 x 5 Latin square presentation of five trial blocks containing five different startle conditions per block : (1) startle stimulus-alone, (2) left LED-startle

stimulus, (3) center LED-startle stimulus, (4) center LED-without startle stimulus and (5) right LED-without startle stimulus. The order of trials within the Latin square was randomized but some adjustments were made to prevent the consecutive appearance of two of the same trial conditions and the repetition of more than three startle conditions in a row. Intervals between trials were varied from 20 to 60 seconds using no predetermined order of interval lengths. The mean inter-trial interval was approximately 40 seconds. The same Latin square was used for five groups of five subjects each (N=15). Each subject within a group began at the same row of the square, while each group began at different rows of the square.[2].

The subject was informed at the beginning of the session that (1) onset of the left light would always be followed by a loud tone exactly two seconds later, (2) onset of the center light meant that a subsequent tone might or might not occur and (3) onset of the right light meant that no subsequent tone would occur. Subjects were not informed of the startle stimulus-alone condition. In addition to the information concerning stimulus conditions each subject was instructed to sit quietly and move as little as possible. Each subject was asked to maintain visual fixation on the three lights during the session. All subjects responded affirmatively when asked if the instructions were completely understood.

At the completion of the session each subject was given a brief description of the study. Any questions the subjects asked were answered as well as possible. The subjects were asked not to divulge any information concerning the study to any prospective subjects.

Response Recording

EMG. Muscle potentials,or the electromyogram (EMG), for the first seven subjects were recorded from the skin surface below the lower eyelid using Grass gold electrodes. Beckman miniature silver-silver chloride biopotential electrodes and adhesive collars were used for the last eight subjects to facilitate a more accurate electrode placement. Grass EC2 electrode cream was used as the electrolyte between the skin surface and electrodes. The first electrode was placed 1.0 cm below and 1.0 cm lateral to the nasal terminus of the right eyelid. The second electrode was placed 1.5 cm lateral to the first. Both electrodes were placed above the muscle controlling eyelid closure and blink, the orbicularis oculi (Morrison, Cornitt, Tether and Gratz, 1972). EMG was amplified using a Grass P15 A.C. preamplifier. The 1/2 amplitude low frequency filter was set at 30 Hz to filter low frequency potentials generated by eyeball movement from the recording. The high frequency filter was adjusted to 300 Hz. The frequency range 30 to 300 Hz covers the largest portion of the EMG activity spectrum (Goldstein, 1972). The EMG signal from the Grass preamplifier was again amplified by a shop-built D.C. amplifying circuit and recorded in analog form on magnetic tape using channel 2 (response frequency range: 0 to 300 Hz) of the Vetter C-4 tape recorder. In addition to the EMG data, a 400 microvolt (uv) peak-to-peak signal from a Grass SWC-1 square wave calibrator, amplified and filtered by the same EMG recording system, was included on the same tape channel to provide a means of calibrating tape recorder output and determining EMG response amplitude.

EKG. Analog EKG was recorded using Beckman biopotential cup electrodes

and EKG sol as the electrolyte. Each electrode was attached to the volar surface of either forearm with Beckman adhesive collars. EKG was amplified and filtered at 1 and 100 Hz 1/2 amplitude low and high frequencies, respectively, using a Grass P15 preamplifier. The EKG was then recorded directly on to FM tape,using channel 3 of the Vetter recorder, for later reduction to heart period and heart rate.

EEG. Using two Beckman miniature biopotential electrodes and Grass EC2 electrode cream, EEG was recorded for later analysis of the contingent negative variation (CNV). The vertex was used as the active site and the right earlobe as reference. A 200 uv square wave signal from the Grass SWC-1 calibrator was recorded on the EEG channel prior to application of the EEG electrodes to calibrate the tape recorder output gain and to determine CNV amplitude.

Response Measurement

EMG. Each tape recorded EMG response was first rectified by a shop-built full wave rectifying circuit. With the onset of the taped trigger pulse synchronized with startle stimulus onset, the rectified EMG signal was sampled twice/ms for 128 ms by a Tracor-Northern 560 Signal Averager with an 8-bit binary analog-to-digital converter (ADC). The output of the tape recorder was adjusted so that each subject's largest response used nearly all of the ADC's range to optomize the signal-to-noise ratio for all the startle responses.

A program was written to input the digitized EMG values to the calculator and to score each response for latency and amplitude. From inspection of oscilloscope photographs of all 225 EMG responses it was determined that no response began sooner than 20 ms after startle

stimulus onset. The calculator determined the largest deviation in this baseline period and then searched for the first digital value which exceeded this deviation score by four counts (See Appendix 1). Once the onset of the response had been determined all the digital values up to that point were used to calculate an average baseline level of EMG activity for that particular response.

Beginning with the response latency point and continuing for the next 40 ms, the amplitude for each address in the signal averager memory was summed and averaged by the calculator. Response amplitude was calculated by subtracting the average baseline amplitude from the 40 ms response window average amplitude. Excessive baseline noise resulted in the elimination of one EMG response from the study. The latency and amplitude means of that subject's set of scores for that particular startle condition were used to replace the two missing scores.

The average response amplitude as determined by the calculator was subsequently converted to average microvolts/address using the 400 uv EMG calibration signal. Latency was measured to the nearest 0.5 ms. Both latency and amplitude scores were converted to standard z-scores for statistical analyses.

EKG and Heart Rate. Beginning with the first taped trigger pulse the calculator timed 16 consecutive R-R intervals. It then calculated and printed heart rate in beats per minute for each of four pre- and fifteen post-lead stimulus 200 ms intervals. Therefore, in all startle stimulus conditions, heart rate was divided into fourteen pre-startle and five post-startle intervals. When an R-wave occurred during one of the 200 ms intervals the heart rate for that interval represented a

proportional, weighted value of two consecutive heart beats.

The heart rate of the fourth 200 ms interval occurring prior to lead stimulus onset was used as a baseline level of heart rate for each trial. The heart rate of the fourteenth interval which is the interval preceding startle onset was subtracted from the baseline heart rate value to provide a deceleration score for that trial. Also, a score for heart rate acceleration was obtained by subtracting the heart rate of the fourteenth interval from that of the last 200 ms interval.

CNV. For each trial beginning with the taped trigger pulse, analog EEG was sampled once every 10 ms for 5120 ms by the signal averager. Five trials per condition were averaged and converted by the digital-to-analog converter (DAC). The output of the DAC was plotted on graph paper using a Hewlitt-Packard 1035B X-Y Plotter. Five separate curves were obtained for each subject. Each curve was hand-scored for amplitude every 256 ms from approximately 848 to 80 ms prior to lead and startle stimulus onsets. The four amplitudes preceding the lead stimulus were averaged to provide a baseline EEG score. This average was then subtracted from the average of the four EEG scores obtained prior to startle stimulus onset. This difference provided a single CNV score for each of the startle conditions.

A calculator program collapsed digitized EEG amplitudes to test for possible differences in CNV in the 100% and 50% startle certainty conditions. EEG was averaged into 14 consecutive 150 ms intervals beginning 150 ms before lead stimulus onset and continuing until 50 ms before startle stimulus onset. The first interval represented a base-

line EEG score and was subtracted from each of the following intervals. The next three intervals after the baseline period covered the length of the evoked response to the lead stimulus. The last ten intervals were used to score CNV in the period preceding the startle stimulus. The interval in which the largest positive deviation from the baseline EEG amplitude occurred was scored as latency to peak CNV amplitude.

Results

EMG Response to the Startle Stimulus

Latency of the EMG startle response was significantly facilitated by the presence of a lead stimulus ($F(2,8)=83.8$, $p<.001$, Table 1). Both the 100% (high certainty) and 50% (low certainty) startle latency means were significantly shorter than the average response latency of the control startle condition (Figure 1). In addition, the EMG response latency was significantly faster in the high certainty condition than in the low certainty condition.

As predicted, uncertainty in the lead interval elicited significant startle amplitude facilitation in the low certainty condition ($F(2,8)=9.16$, $p<.01$, Table 2). Z-score amplitude means x 100 appear in Figure 1 for each of the startle response conditions. Amplitude of the EMG response in the high certainty condition was not significantly greater than the control startle response. The low certainty startle responses were significantly larger than both the control startle responses ($F(1,75)=4.22$, p .01) and the high certainty responses ($F(1,75)=2.43$, $p<.05$).

Responses Preceding the Startle Stimulus

Heart Rate. Cardiac changes in beats per minute for each of the nineteen consecutive 200 ms intervals is presented in Figure 2 for each of the five experimental conditions. All lead stimulus conditions, including the 0% startle probability condition, demonstrated significant cardiac

deceleration ($F(4,16)=4.80$, $p<.01$, Tables 3 and 4). Habituation of the deceleratory response with repeated presentations of conditions did not occur (trials $F(4,16)=1.52$).

Heart rate acceleration also varied with startle conditions following startle onset ($F(4,16)=8.73$, $p<.01$, Table 5). Analysis of the acceleration means indicated significantly larger accelerations in both the low certainty (with startle stimulus) and high certainty conditions than in the control startle condition (Table 6, Figure 3). On those low certainty trials not followed by a startle stimulus heart rate continued to decelerate following the point in time during which the startle stimulus would have occurred (Figures 2 and 3).

CNV. The hand-scored EEG amplitude in the interval following the lead stimulus showed significant CNV development in all lead-startle stimulus conditions ($F(4,16)=10.72$, $p<.01$, Table 7). CNV was also present, of course, in the low certainty condition without a startle stimulus since it was found in the paired low certainty condition (Figure 4). Although no significant CNV was found following the 0% startle warning stimulus (Table 8), a late negative shift in the evoked response to this lead stimulus can be seen in Figure 4.

Using the collaspsed 150 ms EEG scores no significant conditions x interval interaction was found to indicate that the shape of the cortical evoked response to the lead stimulus was different for the high and low certainty conditions. However, the shapes of the two CNV curves using the last ten 150 ms intervals (Figure 5) were found to be significantly different (conditions x interval $F(9,36)=2.78$, $p<.025$, Table 9). The shape of the CNV in the low certainty condition is charac-

terized by a rapid rise to peak negativity, while the CNV following the the high certainty lead stimulus rises gradually to peak amplitude.

Discussion

The effects of varying startle stimulus probability on the amplitude and latency of the EMG response are summarized in Figure 1. As predicted the low startle certainty condition facilitated both EMG amplitude and latency. Thus, EMG as a measure of startle replicates the startle blink facilitation found in previous uncertainty paradigms. Similarly, Bloch (1975) has replicated the short lead interval inhibitory effect using the integrated EMG response. The latencies of the response in the current study ranged from 20 to approximately 50ms and were much faster than blink response latencies of 46 to 110ms (Bloch, 1972). In any case, while differences in response latency may reflect different response systems, the replication of inhibitory and facilitory effects indicates that the same processes mediating lead stimulus modulation of blink startle are modulating the EMG response as well.

While previous studies have produced uncertainty and startle facilitation by varying lead interval length in combination with either various lead stimulus durations or terminal stimulus intensities, the current low certainty condition held lead stimulus duration and interval constant. Equivocation of the startle stimulus was the only source of uncertainty in the lead interval. This extends the types of time-locked uncertainty capable of producing the facilitation effect

and confirms that phasic uncertainty in general rather than any particular source is sufficient to produce startle facilitation.

Consistant with this view, the absence of uncertainty in the high startle certainty condition did not elicit significant amplitude facilitation. However, startle latency was significantly facilitated, contrary to the findings of earlier high certainty paradigms (Bloch, 1972; Brown, 1975). This high certainty latency facilitation is thus independent of interval or terminal event uncertainty. Furthermore, the high certainty condition also elicited more latency facilitation than the low certainty condition. This suggests that mediators of the high certainty latency facilitation were not necessarily identical to the mediators of the low certainty facilitation effects. The fact that significant amplitude facilitation accompanied the low but not the high certainty facilitation also supports this suggestion. Further, this uncoupling of amplitude and latency effects also indicates that mediators of latency facilitation are not all shared by processes facilitating amplitude.

The presence of heart rate deceleration and CNV in the high certainty condition also runs contrary to earlier findings. This may be due to contextual differences in which the high certainty conditions were embedded. Both the Bloch (1972) and Brown (1975) studies contained high certainty paradigms in which lead stimulus detection signaled that a startle stimulus would follow 100% of the time. No lead stimulus discrimination was required in the paradigm. However, onset of the high certainty lead stimulus in the present study required not only that it be detected but also discriminated from other lead stim-

uli representing different startle probabilities. Orienting and attentional/arousal processes reflected in cardiac deceleration and CNV, elicited by lead stimulus onset, thus may be maintained by the discrimination task. This is supported by the presence of both heart rate deceleration and an apparent late evoked potential negativity (Figure 4) associated with orienting (Loveless and Sanford, 1975), which follows the onset of the 0% startle lead stimulus. Other differences between the current and earlier studies, such as instructions and multiple versus single lead stimuli, only reinforce the importance of the initial discrimination.

In previous studies, conditions which produce startle facilitation also produced heart rate deceleration and CNV. This also held true in the present study. However, significant cardiac deceleration accompanied each of the three lead stimulus conditions and did not differentiate one condition from another. Nor did CNV amplitude preceding the startle stimulus vary as a function of high versus low certainty. Further, heart rate deceleration and CNV amplitude failed to correlate significantly with either startle amplitude or latency facilitation. Differences in EMG startle amplitude or latency between the high and low certainty conditions cannot, therefore, be explained by the processes mediating the heart rate deceleration or CNV amplitude.

The offset of orienting and attentional processes caused by startle stimulus onset has been proposed by Graham (1975) to produce a physiological "rebound" which leads to startle facilitation. While only one second of post-startle heart rate was analyzed, greater heart rate acceleration was present in the two warned startle conditions

than in the control startle condition. The two warned conditions, however, did not differ from each other. Correlations between heart rate acceleration and latency facilitation were not significant. This indicates that a rebound effect cannot account for startle amplitude facilitation in the low certainty condition and its absence in the high certainty condition. Nor can rebound account for the shorter response latencies in the high certainty condition than in the low certainty condition. Thus there is little evidence for the suggestion that responses associated with the termination of orienting and attentional processes can adequately account for the differential startle facilitation effects obtained in the present study.

Although only marginally significant, there is a tendency for the high and low certainty conditions to differ in CNV shape. Figure 5 shows a monotonically rising CNV shape in the high certainty condition, while the curved shaped CNV occurring in the low certainty condition is similar in form to a Type A CNV which has been previously associated with interval uncertainty (McAdam, 1969; McAdam, Knott, and Rebert, 1969). Thus, while CNV amplitude does not distinguish the high and low certainty conditions, mechanisms involved in the development of CNV shape do appear to be related to the differences found between the two conditions. In fact, EMG amplitude facilitation and CNV latency to peak amplitude correlated significantly in the expected direction (Pearson's $r = -.47$, $p < .05$, one-tailed test). Bloch (1972) also reported a curved CNV shape accompanying startle facilitation, but did not find a significant correlation between CNV latency to peak and amplitude facilitation (1975), which may reflect differences in experimental designs

or the inadequacy of CNV latency to peak amplitude in reflecting CNV shape. Thus startle amplitude facilitation and CNV shape may share a common mediator. It may also be the case that the same process is responsible for the difference in startle latency facilitation between the high and low certainty conditions.

The differences in CNV shape indicate different distributions of cortical activity accompanied the differential startle effects. Further, the different sources of arousal inherent in the experimental design may account for the different distributions. In other words, discrimination among lead stimuli may produce a different distribution of cortical arousal than uncertainty as to startle stimulus occurrence. The discrimination among multiple lead stimuli appears sufficient to mediate startle latency facilitation as well as the heart rate deceleration associated with orienting. In a paired stimulus condition the distribution of cortical arousal induced by the discrimination appears linked to a monotonically rising CNV. The uncertainty as to events following the lead stimulus, such as interval length, terminal stimulus probability or intensity, appears sufficient to mediate startle latency and amplitude facilitation as well as cardiac deceleration. Further, terminal stimulus uncertainty appears crucial to cortical activity associated with an "A" type CNV.

These speculations suggest that previous paradigms reporting neither latency nor amplitude facilitation effects must have lacked both lead and terminal stimulus variability. This, in fact, was the case. This also accounts for the absence of significant heart rate deceleration and CNV in those paradigms.

A physiological mechanism which is sensitive to the distribution of cortical arousal has been described by Sauerland and his co-workers (Sauerland, Nakamura and Clemente, 1967; Sauerland, Knauss, Nakamura, and Clemente, 1967). They demonstrated that stimulation of the rostral portion of the orbital gyrus located in the prefrontal area, can facilitate or inhibit reflexes via centers in the pons and medulla. In addition to receiving input from all sensory modalities (Korn, Wendt and Albe-Fessard, 1966), the nature of the effects of orbital gyrus stimulation on reflexes was shown to vary as a function of arousal of the surrounding cortical areas. When the surrounding cortex was synchronized, reflexes were inhibited, while cortical desynchrony produced reflex facilitation. Thus different distributions of cortical arousal reflected in CNV shape may differentially affect orbital gyrus activity and mediate the differences in reflex amplitude and latency facilitation between the high and low certainty conditions.

The results of the present study also support the conclusion of Hillyard and Galambos (1967) that equivocation of a terminal stimulus does not reduce CNV amplitude. However, as the current study indicates equivocation may play an important role in determining CNV shape. Thus, studies investigating the effects of S_2 equivocation must consider shape as well as amplitude.

To summarize, The present study demonstrates that the EMG response is modulated by the same processes that modulate the blink component of human startle. Further, the simple equivocation of a startle stimulus was sufficient to elicit facilitation suggesting that complexity

of future studies can be reduced to only two conditions to elicit the facilitory effect, an equivocated startle stimulus condition and a control startle condition. If true, this would simplify the investigation of the processes modulating startle facilitation in difficult populations such as infants and psychotics.

The differences in startle facilitation found in the high and low certainty conditions were suggested to reflect the influence of diferent distributions of cortical arousal on a reflex modifying mechanism located in the frontal cortex. The discrimination among lead stimuli and the uncertainty engendered by startle stimulus equivocation were suggested to be the sources of differential arousal and may have produced the differences in CNV shape.

Appendix 1

This criterion was used to prevent a random noise level of ± 1 binary bit from spuriously adding to the true signal and leading to an incorrect latency or amplitude score. When the first criterion was met the next point was also required to meet the same criterion. If the two successive points were both greater than the largest deviation in the baseline, then the first point determined latency of the response. If the second point was less than four counts higher than the baseline deviation score, then the amplitude of the first point became the new largest baseline deviation. The calculator then began a new search for two successive values at least one count larger than this new baseline deviation until the criterion for latency was met.

Notes

1. Jerger and Tillman (1959) have found the threshold of a 1000 Hz tone to be approximately 6 dB (re 0.0002 microbar) less using the supra-aural cushion (MX 41/AR) than the earshaped circumaural cushion 001A. The supra-aural cushion was used to calibrate the stimulus in the present study, while the circumaural cushion was used during stimulus presentations. This means that the startle stimulus would have been only about 109 dB. However, the acoustic stimulus in the present study is presented binaurally compensating for the loss in sound pressure level.
2. Order of startle stimulus trials is a between groups factor in the present design. Because the order of trials in the Latin square was randomly selected, this between groups factor is considered a random factor and consequently changes the appropriate error terms in the analysis of variance for each dependent variable.
3. Eye movement subsequent to the lead stimulus was not recorded and could have possibly contaminated the amplitude of the CNV response. However, there is no reason to suspect systematic eye movement in the lead interval as is often the problem in the reaction time CNV paradigm. First, subjects were asked to maintain visual fixation on the black box during the session. Secondly, the EEG recording following the 0% startle warning light did not demonstrate any slow negative shift, strongly suggesting that the other lead stimulus conditions were also free of eye movement potentials.

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Table 1

Analysis of the Variance in EMG startle latency z-scores

Source	DF	MS	F
Between subjects			
Order	4	$.21 \times 10^{-4}$	5.17*
S(Order)	10	$.04 \times 10^{-4}$	
Within subjects			
Startle Conditions	2	46.22	83.77***
Order x Conditions	8	.55	<1
S(Order) x Conditions	19	.68	
Trials	4	1.86	3.11*
Order x Trials	16	.60	<1
S(Order) x Trials	40	.70	
Conditions x Trials	8	.45	<1
Order x Conditions x Trials	32	.70	1.64*
S(Order) x Conditions x Trials	80	.43	

* = $p < .05$

** = $p < .01$

*** = $p < .001$

Table 2

Analysis of the Variance in EMG startle amplitude z-scores

Source	DF	MS	F
Between subjects			
Order	4	1.33×10^{-4}	1.30
S(Order)	10	1.02×10^{-4}	
Within subjects			
Startle Conditions	2	7.28	9.16**
Order x Conditions	8	.79	<1
S(Order) x Conditions	19	1.81	
Trials	4	4.25	3.70*
Order x Trials	16	1.15	1.21
S(Order) x Trials	40	.95	
Conditions x Trials	8	.42	<1
Order x Conditions x Trials	32	.90	1.10
S(Order) x Conditions x Trials	80	.81	

Table 3

Analysis of the Variance in heart rate deceleration

Source	DF	MS	F
Between subjects			
Order	4	12.06	<1
S(Order)	10	127.44	
Within subjects			
Startle Conditions	4	231.92	4.80**
Order x Conditions	16	48.27	<1
S(Order) x Conditions	40	57.58	
Trials	4	64.99	1.52
Order x Trials	16	42.64	1.06
S(Order) x Trials	40	40.11	
Conditions x Trials	16	52.19	1.58
Order x Conditions x Trials	64	32.98	<1
S(Order) x Conditions x Trials	160	46.43	

Table 4

Newman-Keuls Analysis of heart rate deceleration means

	Control Startle alone (-.83)	High Certainty without startle (1.92)	Low Certainty without startle (2.16)	High Certainty with startle (3.04)	Low Certainty with startle (3.79)
Control Startle	-	2.75*	2.99*	3.87*	4.62**
High Certainty without startle		-	.24	1.12	1.87
Low Certainty without startle			-	.88	1.63
High Certainty with startle				-	.75
Low Certainty with startle					-

Table 5

Analysis of the Variance in heart rate acceleration

Source	DF	MS	F
Between subjects			
Order	4	73.30	<1
S(Order)	10	86.34	
Within subjects			
Startle Conditions	4	269.31	8.73**
Order x Conditions	16	30.86	<1
S(Order) x Conditions	40	33.37	
Trials	4	47.61	1.05
Order x Trials	16	45.55	2.10*
S(Order) x Trials	40	21.65	
Conditions x Trials	16	7.08	<1
Order x Conditions x Trials	64	25.12	<1
S(Order) x Conditions x Trials	160	25.23	

Table 6

Newman-Keuls Analysis of heart rate acceleration means

	Low Certainty without startle (-.79)	High Certainty without startle (1.50)	Control Startle alone (1.56)	High Certainty with startle (3.50)	Low Certainty with startle (3.79)
Low Certainty without startle	-	2.29*	2.35*	4.29**	4.76**
High Certainty without startle		-	.06	2.00	2.47*
Control Startle alone			-	1.94*	2.41*
High Certainty with startle				-	.47
Low Certainty with startle					-

Table 7

Analysis of the Variance in CNV amplitude

Source	DF	MS	F
Between subjects			
Order	4	70.98	<1
S(Order)	10	258.43	
Within subjects			
Startle Conditions	4	445.44	10.72**
Order x Conditions	16	41.54	1.21
S(Order) x Conditions	40	34.15	

Table 8

Newman-Keuls Analysis of CNV amplitude means

	Control Startle alone (-2.89)	High Certainty without startle (-.10)	High Certainty with startle (7.35)	Low Certainty with startle (7.70)	Low Certainty without startle (9.46)
Control Startle alone	-	2.79	10.24**	10.59**	12.35**
High Certainty without startle		-	7.45**	7.80**	9.56**
High Certainty with startle			-	.35	2.11
Low Certainty with startle				-	1.76
Low Certainty without startle					-

Table 9

Analysis of the Variance in the shape of low and high certainty CNV

Source	DF	MS	F
Between subjects			
Order	4	602.37	<1
S(Order)	10	1417.13	
Within subjects			
Startle Conditions	1	362.78	1.57
Order x Conditions	4	230.62	1.63
S(Order) x Conditions	90	13.28	
Intervals	9	147.69	7.53***
Order x Intervals	36	19.60	1.47
S(Order) x Intervals	90	13.28	
Conditions x Intervals	9	23.83	2.78**
Order x Conditions x Intervals	36	8.56	<1
S(Order) x Conditions x Intervals	90	15.24	

** = $p<.025$ for this analysis only

. Figures

1. Amplitude and latency of EMG responses as a function of lead stimulus condition.

2. Heart rate changes preceding and following startle stimulus onset for each lead stimulus condition. Each point represents the average of 200 ms and is plotted at the end of the interval.

3. Heart rate acceleration and deceleration as a function of lead stimulus condition.

4. EEG activity averaged over subjects beginning 4 seconds before startle stimulus onset and continuing for 1.12 seconds after startle stimulus onset.

5. EEG changes following lead stimulus onset in high and low startle certainty conditions. Each point represents the average of 150 ms and is plotted at the end of the interval.

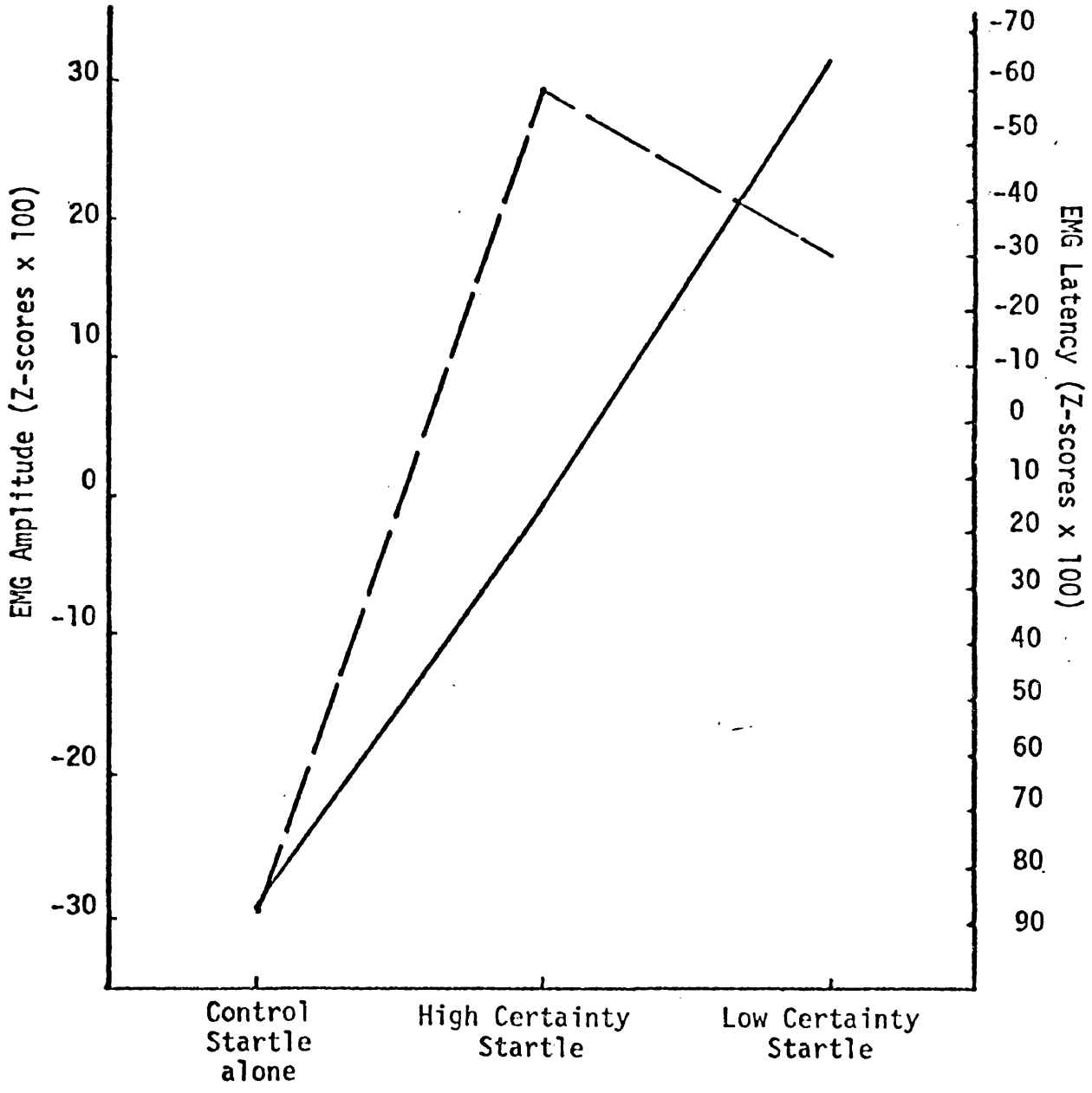
EMG Amplitude (Z-scores x 100)
30
20
10
0
-10
-20
-30
EMG Latency (Z-scores x 100)
-70
-60
-50
-40
-30
-20
-10
0
10
20
30
40
50
60
70
80
90
Control Startle alone
High Certainty Startle
Low Certainty Startle

Figure 1

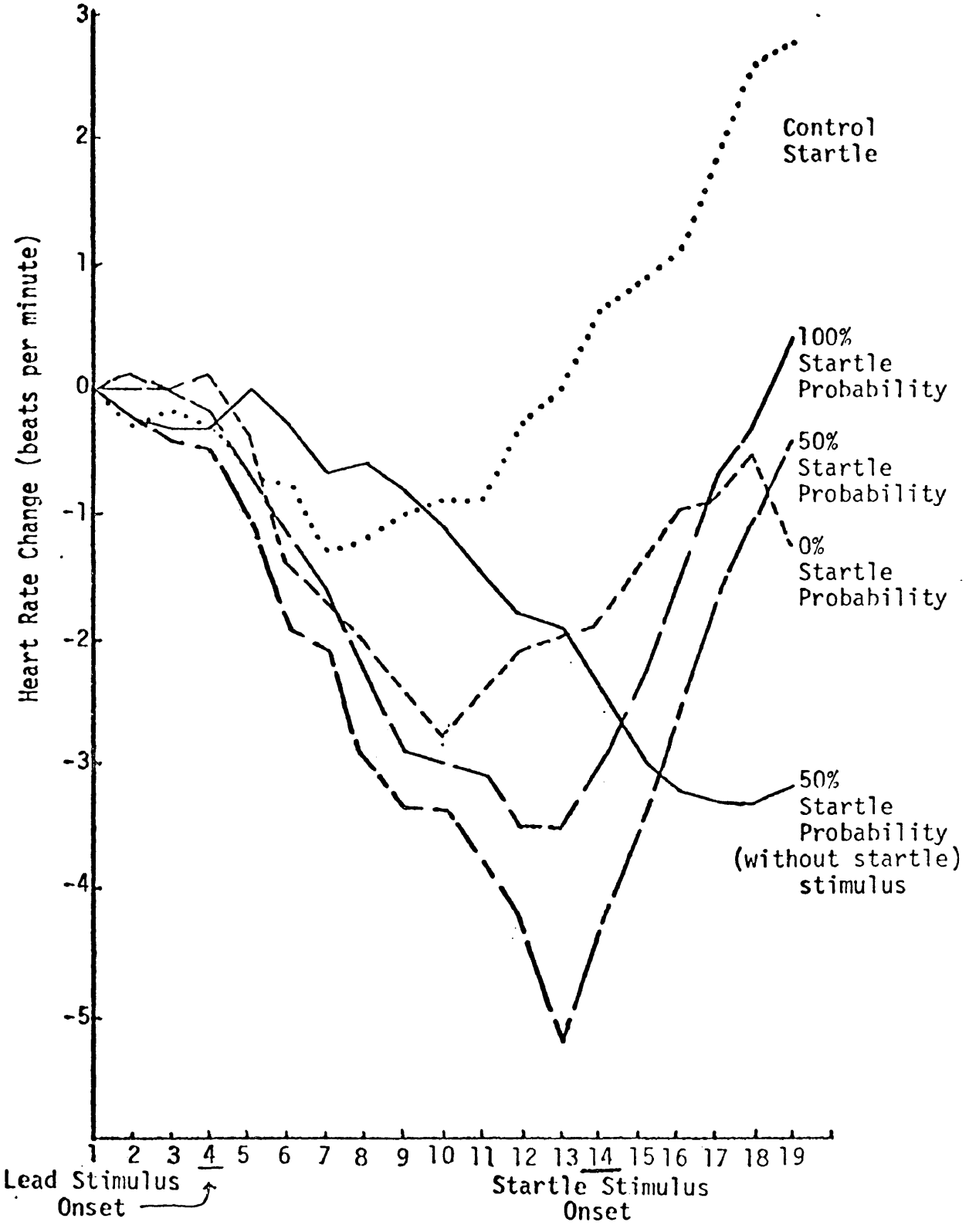
Heart Rate Change (beats per minute)
3
2
1
0
-1
-2
-3
-4
-5
Control Startle
100% Startle Probability
50% Startle Probability
0% Startle Probability
50% Startle Probability (without startle) stimulus
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Lead Stimulus Onset
Startle Stimulus Onset
200 ms Intervals

Figure 2.

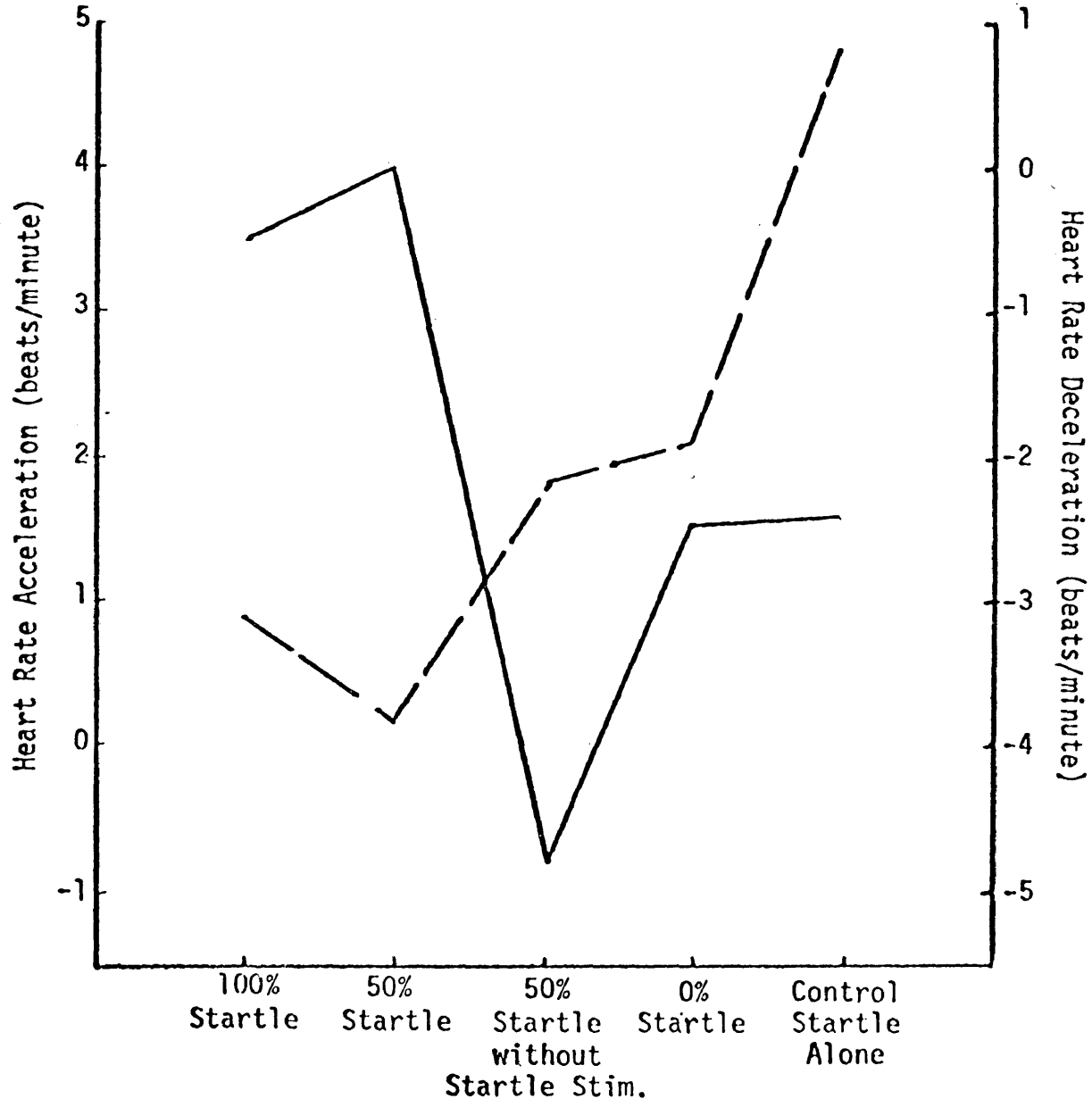
Heart Rate Acceleration (beats/minute)
5
4
3
2
1
0
-1
Heart Rate Deceleration (beats/minute)
1
0
-1
-2
-3
-4
-5
100% Startle
50% Startle
50% Startle without Startle Stim.
0% Startle
Control Startle Alone

Figure 3.

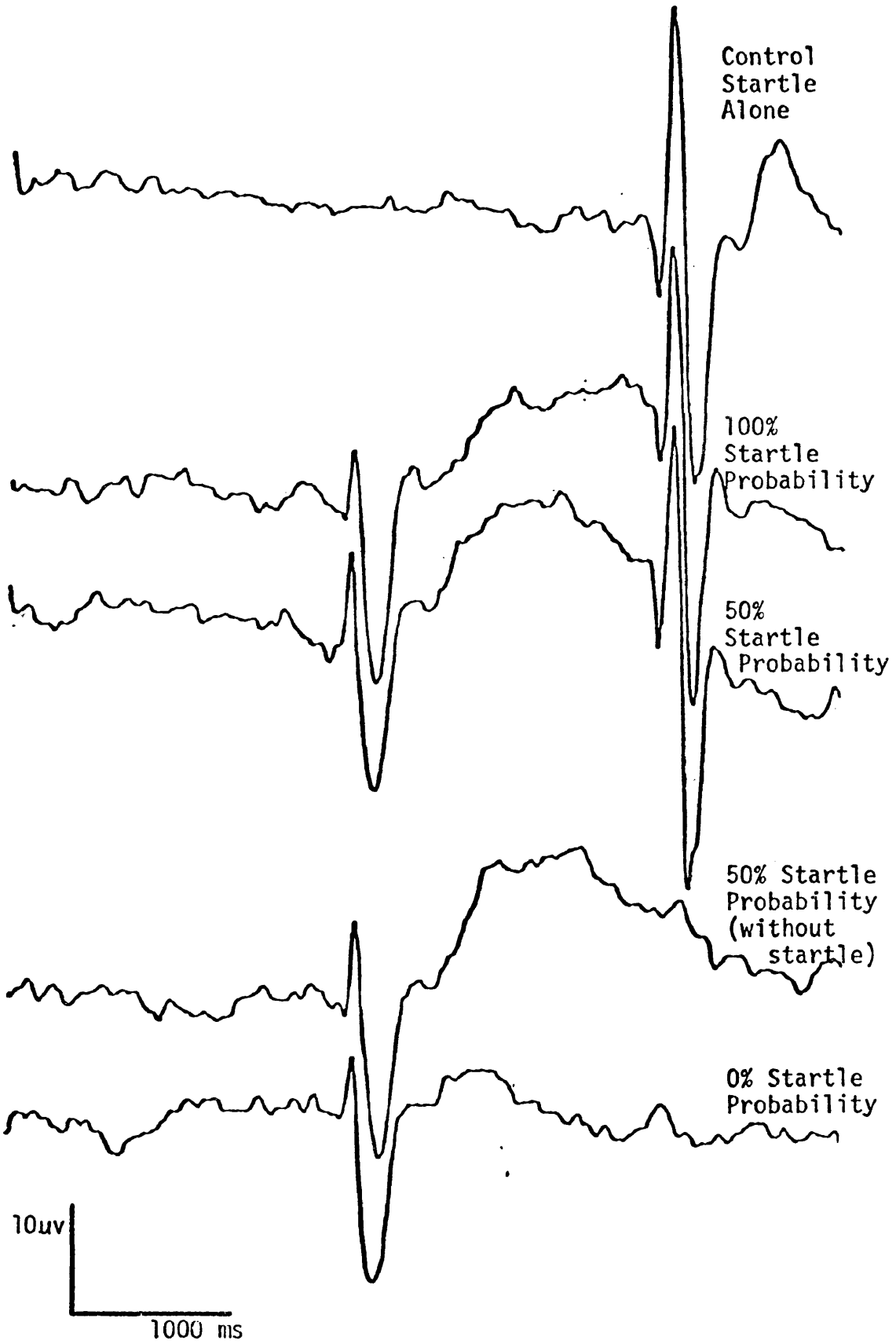
Control
Startle
Alone
100%
Startle
Probability
50%
Startle
Probability
50% Startle
Probability
(without
startle)
0% Startle
Probability
10uv
1000 ms

Figure 4.

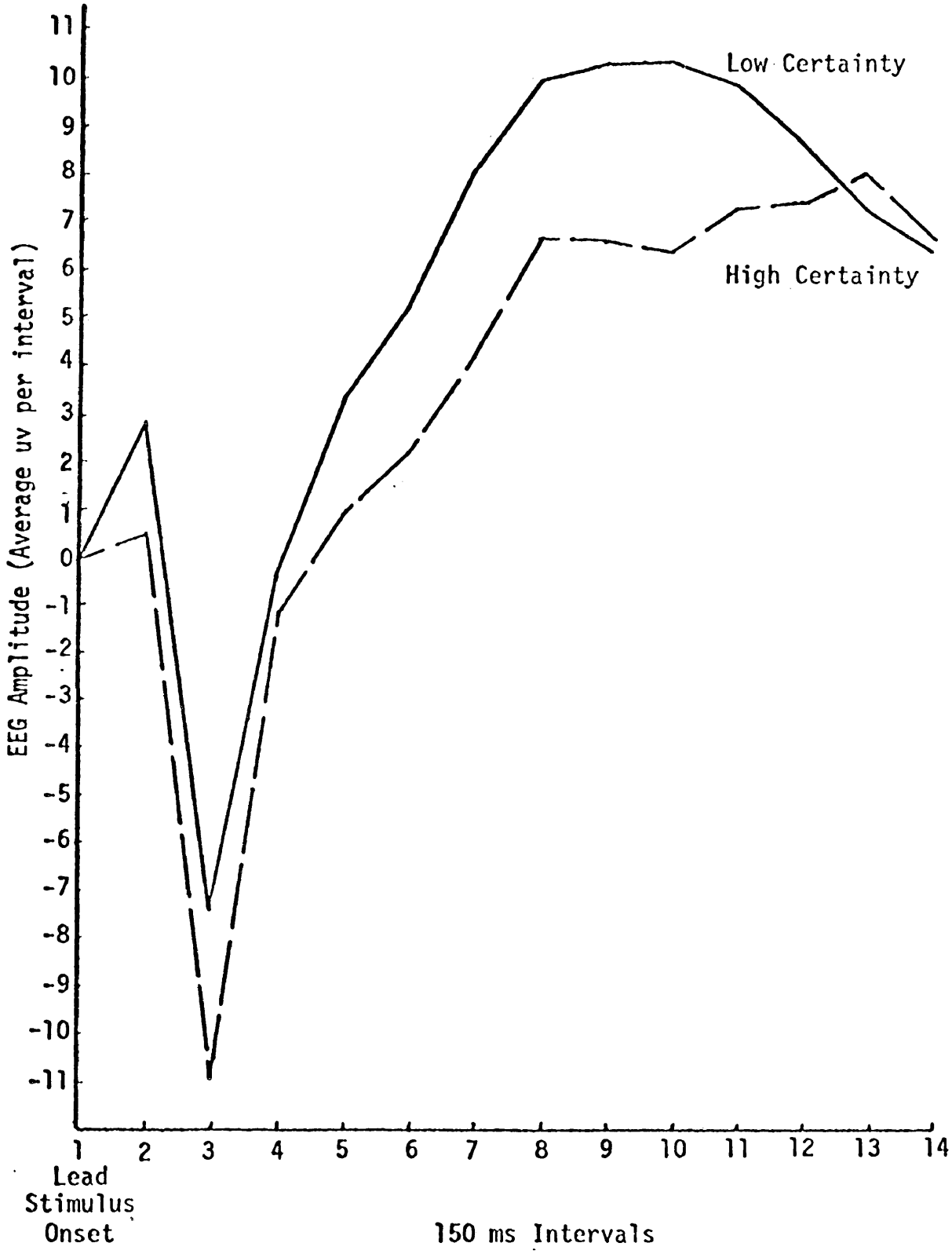
Low Certainty
High Certainty
EEG Amplitude (Average uv per interval)
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Lead Stimulus Onset
150 ms Intervals

Figure 5

VITA

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