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Startle response probability and amplitude may be independently modulated by affective foreground stimulation as acoustic probe intensity decreases

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Startle Response Probability and Amplitude may be Independently Modulated by Affective Foreground Stimulation as Acoustic Probe Intensity Decreases

The magnitude of the eyeblink reflex to an acoustic startle probe is reliable potentiated to highly arousing unpleasant foreground stimuli and inhibited to highly arousing pleasant foreground stimuli across all probe intensity levels. The present study examined the response magnitude findings of Cuthbert, Bradley, and Lang (1996) as response amplitude and probability. Medium arousal pleasant pictures produced larger blink amplitude responses than unpleasant pictures of the same arousal level to 80 and 95, but not 105 dB acoustic startle probes. This effect was opposite for high arousal pictures at all intensity levels. Response probability means decreased from pleasant to unpleasant across all arousal levels to 80 dB probes. The current study provides insight into the differential activation of response amplitude and probability to affective foreground stimulation at lower acoustic stimulus intensities and possible implications for mechanisms involved in the orienting and defensive responses.

The startle response is a diffuse skeletomuscular reflex observed in all mammalian species that directly measures activation of the central nervous system (Davis, 1984, 1992). Neurocircuity of the startle reflex is currently understood in animal models and may have direct translation to studies of human emotion (Lang, Davis, & Ohman, 2000). The human startle response has been used as a reliable measure to investigate attention (Anthony, 1985), emotion (Cuthbert, Bradley, & Lang, 1996), anxiety (Cuthbert et al., 2003), and fear (Grillon & Davis, 1997). However, definitive application of the human startle response paradigm to further investigation and understanding of affective processing and dysregulations associated with psychopathology await parametric replications.

It has been established that the size of the startle eyeblink response varies according to the affective valence (pleasantness) and arousal (activation) of a foreground picture stimuli. The startle response is reliably potentiatied during the viewing of highly arousing unpleasant pictures and inhibited during the viewing of highly arousing pleasant pictures (Cuthbert et al., 1996). Thus, the startle response has become a reliable measure of the affective modulation induced by foreground stimulation (Lang

et al., 1990). Results are theorized of the construct of motivated attention, whereas the human organism is attentively motivated towards appetitive stimulation (e.g. erotica) and attentively motivated to avoid aversive stimulation (e.g. threat) (Lang et al., 1990). Standardized emotionally activating pictures selected from the International Affective Picture System (Lang, Bradley, & Cuthbert, 2005) are used by numerous laboratories as reliable and consistent foreground stimuli for affective modulation.

The startle probe itself generally consists of a sudden, intense acoustic burst of noise presented through headphones (Davis, 1984; Blumenthal et al., 2005). An acoustic startle probe is suggested because researchers are able to manipulate the bandwidth, intensity, rise time, and duration of the acoustic stimulus and subsequently influence the latency, probability, and amplitude of the startle response (Berg & Balaban, 1999; Blumenthal & Berg, 1986; Blumenthal et al., 2005). The acoustic startle stimulus usually consists of a broadband (white) noise with instantaneous rise time presented for 50 milliseconds at 95 decibels (Blumenthal et al., 2005). It is important to note that the acoustic startle stimulus itself does not inflict an emotional state into an individual; the startle probe is simply a way of reliably inducing a startle response to measure activation of the central nervous system, which is modulated by ongoing affective processing that may be induced by foreground stimulation (such as emotional pictures) (Lang et al., 2000).

The startle response may be measured and quantified in various different ways to reflect numerous parametric manipulations (Blumenthal et al., 2005). It is well know that the first and most reliable component of the human startle reflex is the eyeblink response (Landis & Hunt, 1939). Standardized human startle recording techniques have been proposed to promote the replication of results between laboratories (Blumenthal et al., 2005). For instance, it has been strongly suggested that the blink response be recorded with electromyographic (EMG) electrodes placed on the orbicularis oculi muscle directly underneath the eye (Blumenthal et al., 2005). The raw EMG recording is then amplified, filtered to minimize noise, rectified (or made into absolute values), and then integrated (smoothed) (Blumenthal et al., 2005). The startle blink response is generally reported in magnitude values, though important differentiations have been made between startle blink amplitude and probability (Blumenthal & Berg, 1986).

When reporting the size of a single startle response, the terms amplitude and magnitude are interchangeable (Blumenthal et al., 2005). For a set of trials, however, startle response is usually reported as a conditional mean (magnitude), which traditionally includes values of zero for non-response trials (Blumenthal et al., 2005). The mean response magnitude is computed as the product of the mean amplitude and probability for that set of trials ($mM = mA \times P$; Blumenthal & Berg, 1986). Amplitude is computed by averaging startle responses after removing non-response and error trials. Response probability is computed as the total number of responses divided by the total number of startle probes presented after removing error trials (Blumenthal et al., 2005). Most laboratories only report startle response magnitude values, since they are computed by multiplying mean response amplitude and probability.

Individual differences in the baseline of the startle response have been detected when responses are analyzed separately as both probability and amplitude (Blumenthal, et al., 2005). For instance, one participant may be extremely responsive (i.e. highly probable of startling) to the acoustic probe, but produce relatively weak startle responses (i.e. low amplitude startles). On the other hand, another participant may be extremely unresponsive, but produce relatively high amplitude startle responses. It is clear that pooling each of these subjects into a magnitude value for the overall startle response would not accurately represent the individual differences of the startle response (Blumenthal & Berg, 1986). Thus, it is important to differentially interpret and analyze the response probability and amplitude for each participant to ensure that individual differences do not skew the startle magnitude values (Blumenthal, 1996).

Acoustic startle stimulus intensity may also highlight distinctions between startle response probability and amplitude. For instance, it has been demonstrated that increasing stimulus intensity results

in significant changes in startle response amplitude even as response probability approaches 100% (Blumenthal & Berg, 1986). Furthermore, startle responses have been measured at acoustic probe intensities far below "threshold" intensity (85dB; Berg, 1973) by separating response magnitude into amplitude and probability to assess responding below a probability of 50% (Blumenthal & Goode, 1991). Blumenthal and Goode (1991) have suggested that the distinction between amplitude and probability may be more evident at lower stimulus intensities. Findings of the differential response of these two measurements provide support for the suggestion that response probability may reflect a startle "trigger," while response amplitude may reflect a startle "amplifier" (Blumenthal & Berg, 1986). The distinction between a startle response trigger and amplifier has also been supported by suggestions that two independent neural mechanisms are involved in the startle eyeblink response to an acoustic startle probe (Graham, 1979).

Auditory processing involves transient and sustained system activation, which may be somewhat analogous to Y- and X- cells in the visual processing system (Blumenthal & Goode, 1991). The transient system is believed to be more sensitive to auditory stimulus detection, while the sustained system involves processing the continued features of a stimulus. It is argued that the startle response to an acoustic probe is an indicator of transient system activity because of its sensitivity to stimulus rise time (Blumenthal & Berg, 1986). However, the distinction between transient and sustained systems may become more evident at low intensity stimulation (Blumenthal & Goode, 1991). Possible differentiation between these two parallel auditory systems may have an important role in the current human startle response research.

Parallel processing hypotheses of the transient and sustained auditory systems are based on single cell recording of neurons in animal models (Plant & Hammond, 1989). For instance, short time constant (STC) neurons are more sensitive to sudden, low intensity stimuli, whereas long time constant (LTC) neurons may be involved in a more complex analysis of a stimulus. It has been suggested that STC and LTC neurons in the auditory system are relevant to startle responding based upon the distinction between transient and sustained systems (Blumenthal & Goode, 1991). This distinction is more pronounced at the neural firing threshold of both systems, while differences between the two systems decrease to stimulus of high intensity (Plant & Hammond, 1989). Thus, it is possible that acoustic startle stimulation may produce startle response probabilities and amplitudes which vary systematically to the acoustic stimulus intensity.

It is possible that the current human startle paradigms have not completely assessed the consequences of acoustic stimulus intensity manipulation. Blumenthal (1996) argued in favor of reporting both probability and amplitude whenever possible instead of pooling them together as response magnitude. Clearly, this distinction may be much more important for paradigms using low intensity acoustic startle probes. However, it is possible that the distinction between startle amplitude and probability may be evident at above-threshold stimulus intensities. Because of this, current investigations of the human startle response may have overlooked valuable information by reporting startle response scores in only magnitude values.

In order to further investigate the trigger and amplifier model of the startle center (Blumenthal & Berg, 1986), classic research which provides a foundation for the modern understanding of the human startle response should be reexamined. Specifically, the response magnitude findings of Cuthbert et al. (1996) will be calculated as both response probability and amplitude in order to theorize about possible differences in neural mechanisms involved in the startle response. These data are specifically relevant because the experimental design called for the use of low (80 dB), medium (95 dB), and high (105 dB) acoustic probe intensities. Furthermore, the use of emotional pictures which varied in valence (unpleasant, neutral, pleasant) and arousal (low, medium, high) may provide insight into the possible differential affective modulation of response probability and amplitude at each acoustic intensity level.

Consistent with the suggestion of independent startle mechanisms (Blumenthal & Berg, 1986; Manning and Evinger, 1986) it is hypothesized that

startle response probability and amplitude will be independent of the previously reported magnitude findings. It is assumed that this distinction will be most pronounced for low intensity acoustic probes. Furthermore, the affective modulation produced by foreground stimulation varying in valence and arousal may be differentially correlated with startle response probability and amplitude. Also, the affective modulation of highly arousing emotional pictures observed in the startle response magnitude values may not be reflected in the response amplitude and probability.

Method

Participants

Participants were 70 introductory psychology students (32 females) who participated for course credit. Data from four subjects were not used due to apparatus problems. For further clarification of the precise methods in the original experiment, consult Cuthbert et al. (1996).

Materials and Design

Fifty-four color photographs were selected from the International Affective Picture System (IAPS), a database of photographs depicting various events that are normatively rated for valence and arousal (Lang et al., 2005). Eighteen pictures were selected to represent each of three valence categories (pleasant, neutral, unpleasant). Within each valence category, six pictures represented low-, medium-, and high-rated arousal. The pictures were presented in two blocks of 27 pictures, so that each block included 3 slides of each of the 9 valence-arousal combinations. Valence and arousal categories were used as within-subjects factors for the two-way analysis of variance.

The acoustic startle stimulus was composed of white noise with instantaneous rise time and presented over headphones for 50-ms. Each subject was randomly assigned to each of two startle intensity categories. Thirty-seven subjects received startle probe intensity that was counterbalanced as 80 dB in one block and 105 dB in the other block. The additional 33 subjects received a startle probe intensity of 95 dB during both blocks. A single startle probe was presented at random 2.5-5 s after picture onset during 18 of the 27 pictures in each block, so that a probe was presented during 2 of the 3 slides at each valence-arousal combination. Six startle probes were also presented during inter-picture intervals in each block to enhance unpredictability.

Physiological Recording Apparatus

A Coulbourn S75-01 bioamplifier with a bandpass of 90-1 KHz was used to amplify the electromyogram (EMG) signal. Orbicularis oculi recordings were obtained with two miniature silver/silver chloride electrodes filled with electrolyte paste placed directly below the non-dominant eye. The signal was filtered with a Coulbourn S76-01 contour-following integrator with a time constant of 125 ms. From 50 ms before until 300 ms after the acoustic startle probe onset, the blink response was sampled at 1000 Hz. Response trials with clear artifacts (e.g. movement) or excessive baseline activity were rejected as errors (approximately 6% of all trials).

Images were displayed using VPM stimulus control software (Cook, 2001) running on an IBM computer. Data was controlled by a separate IBM computer running VPM software.

Procedure

After the informed consent procedure, electrodes were attached while the subject was situated in a reclining chair in front of a screen on which the images were presented. The subject was instructed to focus on the image for the entire presentation time (6 seconds). Also, they were told to ignore the occasional noises presented over the headphones. The subject was alone in the room during the presentation procedure while physiological signals were recorded. After completion of the experiment, electrodes were removed and the subject was debriefed.

Data Analysis

The startle response probability was computed by dividing the number of responses by the total number of startle probes, after excluding error trials (Blumenthal et al., 2005). To compute the response amplitude means, missing (non-error) cells were

estimated for subjects where 3 or fewer cells were missing (and not more than one per valence). This was done by dividing the total startle response mean for each intensity level (low, medium, high) by the subject mean in order to determine each subject's individual differences in startle response baseline. Once this percentage was computed for each subject's missing response amplitude, the cell means (e.g. unpleasant x low arousal) for each intensity level were computed and multiplied by the individual subject's mean baseline difference. Subjects who produced a startle response to six or less of the nine startle probes were excluded from the amplitude analysis altogether.

As per Cuthbert et al. (1996), a two-way analysis of variance of the mean startle amplitude and probability values was conducted with valence (three levels: pleasant, neutral, unpleasant) and arousal (three levels: high, medium, low) as within-subject factors. Linear and quadratic trends within these analyses were noted to assess effects across each valence and arousal level. Further analyses were conducted within each level to explore interaction effects. To control for heterogeneity of the covariance matrix, multivariate test statistics were used for all analysis (Vasey & Thayer, 1987).

Results

The three by three analysis of variance for the amplitude values produced a significant interaction between picture valence and arousal for all intensity levels (all F's > 4.65, all p's < .01). This indicates that each picture valence level produced a significant change in amplitude response for each arousal within valence level. There were no significant probability interactions of valence and arousal. The significant interaction of amplitude values was broken down for each valence and arousal category and intensity level to further assess interactions within each of these variables.

At high acoustic probe stimulus intensities, a significant valence effect was observed for highly arousing pictures (F [2,35] = 9.66, p <.001). This result indicates that startle amplitude values increased from pleasant to unpleasant valence categories. Medium probe stimulus intensities also produced a valence effect for both medium (F [2,31] = 7.47, p < .01) and low (F [2,31] = 4.303, p < .05) arousal pictures. However, these results indicate that startle amplitude values decreased from pleasant to unpleasant valence categories. This same effect was also observed at low stimulus intensities to medium arousal pictures (F [2,26] = 5.501, p < .05). No significant valence effects were observed for startle probability values.

Arousal effects were only obtained at high probe stimulus intensities for the unpleasant valence category (F [2,35] = 7.57, p < .005). This indicates that startle amplitude values increased from low to highly arousing unpleasant pictures. Both low and medium intensity probes produced a significant effect of arousal at all valence categories (p < .05). The arousal effects of low and medium intensity probes were stronger for pleasant and unpleasant valence categories (p < .01). These findings indicate that low and medium intensity probes produced startle amplitude values that decreased from low to highly arousing pleasant pictures and increased from low to highly arousing neutral and unpleasant pictures. No significant arousal effects were observed for startle probability values. However, inspection of the probability means (see Table 2) indicates that response probability values increased from pleasant to unpleasant valence across all arousal levels.

Both linear and quadratic trends were significant for each valence by arousal analysis (p < .05). The linear trend indicates that unpleasant pictures of all arousal levels significantly differed from pleasant pictures of equal arousal level. Quadratic trends indicate that unpleasant and pleasant pictures differed from neutral pictures of equal arousal levels. Significant linear trends were observed for each significant valence and arousal effect at all probe intensity level except the arousal effect of pleasant valence at low intensity. This effect is most likely attributed to the significant quadratic trend (F [1,27] = 27.8998, p < .0001). This result indicates that medium arousing pleasant pictures produce a startle magnitude response that is significantly larger than both low and high arousal pleasant pictures. No significant linear or quadratic trends were observed for startle response probability values.

Discussion

Results of the current study indicate that startle response amplitude and probability may be independently modulated by affective foreground stimulation that ranges in valence (pleasantness) and arousal (motivational activation) as acoustic startle stimulus intensity decreases. Low and medium probe intensity levels produced significant linear trends for medium arousal pictures, which indicate that startle response amplitudes reliably decreased from pleasant to unpleasant pictures. Response probability means decreased from pleasant to unpleasant pictures at all arousal levels to low intensity startle probes. Probability means also decreased from pleasant to unpleasant pictures at medium and high arousal levels for medium intensity probes.

Mean startle response probability analyses failed, however, to produce any significant results, possibly because the probability data were not normally distributed. The logit transformation that could be used to normalize the data was not available in this occasion because probability means were restricted to three values (0, .5, 1) for low and high intensity stimulus and seven values (0, .25, .33, .5, .66, .75, 1) for medium intensity probes because each valence by arousal category had the possibility of only two or four startle probes, respectively. Nonetheless, Table 2 indicates the general trend of mean probability values at each valence by arousal category and intensity level. Figure 3 illustrates the likeliness of probabilities at low stimulus intensity to decrease from pleasant to unpleasant pictures for all arousal levels. Also, this figure indicates that low arousing pictures produce the most probable blink responses across all arousal levels at low stimulus intensity.

An interesting finding in the current study is the valence effect observed at medium arousal pictures to 80 and 95 dB startle probes. These findings are contrary to the affective modulation of the startle response (i.e. larger startle amplitudes for unpleasant than pleasant) observed for highly arousing pictures of all probe intensities. Figure 2 indicates that startle amplitudes to medium intensity probes decreased from pleasant to unpleasant at medium and low arousal levels. These results were not significant in the startle magnitude analysis of Cuthbert et al. (1996) and could reflect fundamental differences between response amplitude, probability, and magnitude. It is possible that the startle amplitude is a more direct index of the attentional activation associated with moderately arousing pleasant and unpleasant pictures that produce an equal inhibition of the startle reflex.

For instance, a region of low to medium arousal ratings produced a progressive blink inhibition for both unpleasant and pleasant pictures that was almost identical. This finding leads to the hypothesis that an orienting mechanism is similarly activated by moderate levels of arousal for both categories of affective pictures. The orienting response is associated with an approach disposition, which creates augmented attention and decreased motor activity, thus inhibiting the startle response. It has been suggested that lower stimulus intensities are more likely to produce an orienting response (Sokolov, 1963), and these data support this claim.

Probability mean results also provide support for the increased attentional activation of moderately arousing pictures at each valence level. Figure 3 illustrates that startle response probability means decreased from pleasant to unpleasant pictures for all arousal levels to low intensity probe stimuli. This trend suggests that at low intensity startle probe stimulation, unpleasant pictures induced the most attentional activation and motor constraint, which subsequently inhibited the probability of the startle response. Interestingly, this effect was also observed for highly arousing unpleasant pictures. These findings indicate that lower acoustic probe intensity levels are unable to produce the defensive response associated with the viewing of highly arousing unpleasant pictures. Furthermore, this demonstrates that response probability may be a reliable indicator of the attentional activation induced by unpleasant affective foreground stimuli that is independent of the defensive response. However, firm conclusions cannot be drawn because of the low number of probability trials and the lack of any statistically significant results.

There were several limitations in the current investigation which may have impacted the

generalizability of the results. The original experiment was conducted as an investigation of the effects of picture arousal on previously established valence modulations; varying intensity levels were not experimentally designed to produce reliable effects across conditions. The counterbalancing of low and high intensity probe stimuli may not allow a large enough sample size for an adequate comparison to medium intensity results. Also, more subjects were excluded from the low intensity group for the response amplitude analysis because of the reduced probability of startle to low intensity probes. Future investigations should produce a design with a single participant sample assigned to each intensity condition to avoid any possible effect of stimulus counterbalancing observed in the current study.

Another limitation of this investigation may arise from an idiosyncratic characteristic observed in the medium arousal unpleasant pictures. Cuthbert et al. (1996) recorded skin conductance response to measure activation of the sympathetic nervous system, which correlates strongly with reported arousal and is independent of valence. They reported a slight dip in skin conductance response for medium arousal unpleasant pictures, which indicates that the particular sample of moderately arousing unpleasant stimuli chose in this study may have in fact been less arousing than the sample of moderately arousing pleasant stimuli. It will be important for future investigations to carefully match the sympathetic activation of each arousal category to avoid possible inconsistencies of moderately arousing unpleasant pictures observed in the current study.

There were no statistically significant startle response probability effects because of the restriction of possible probability values. Further investigation into startle response amplitude and probability would require a paradigm that includes a larger number of startle probes for each condition. Counterbalancing of foreground stimulus would be very important to avoid habituation effects. Even so, it is challenging to account for habituation effects of the relatively larger amount of startle probes required per valence by arousal category for each subject trial. More than likely, it may be necessary to include a set of trials over an extended period of time (e.g. consecutive days) for each subject, which will also protect against habituation effects and provide insight into individual differences of startle baseline and amplitude.

Results of the current study support the importance of a standardized methodology for human startle eyeblink investigation. It has been demonstrated that response probability, amplitude, and magnitude may be independently activated by foreground stimulation. Furthermore, the influence of acoustic startle stimulus intensity proved to differentially manipulate startle response probability and amplitude. It is clear that interpretation of the startle response may reflect the parameters in which the startle response is measured and analyzed. Future investigations of the human startle response should consider reporting response values as amplitude and probability to investigate possible independent activations of these two response parameters.

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Table 1

Mean startle response amplitude for each valence by arousal category and intensity level

	Valence								
	Pleasant			Neutral			Unpleasant		
Intensity	Low Arousal	Medium Arousal	High Arousal	Low Arousal	Medium Arousal	High Arousal	Low Arousal	Medium Arousal	High Arousal
Low	234.52	260.75	156.12	185.95	293.73	243.64	240.90	160.67	302.00
Medium	262.31	287.41	186.44	223.89	271.06	278.35	237.03	207.85	303.78
High	643.57	655.23	534.38	676.50	660.61	665.95	630.19	611.49	688.14

Table 2

Mean startle response probability for each valence by arousal category and intensity level

	Valence								
	Pleasant			Neutral			Unpleasant		
Intensity	Low Arousal	Medium Arousal	High Arousal	Low Arousal	Medium Arousal	High Arousal	Low Arousal	Medium Arousal	High Arousal
Low	0.865	0.797	0.792	0.824	0.784	0.811	0.806	0.730	0.764
Medium	0.956	0.960	0.945	0.944	0.928	0.936	0.960	0.944	0.814
High	0.985	1.000	1.000	0.929	1.000	1.000	0.985	1.000	0.985

Figure Captions

Figure 1. Arousal effects across valence levels to medium intensity probes. This graph highlights the significant linear trend of high arousal stimuli amplitude means to increase from pleasant to unpleasant valence and medium arousal amplitude means to decrease from pleasant to unpleasant.

Figure 2. Medium arousal effects of valence at each probe intensity level. This graph illustrates the significant linear trend of amplitude means to decrease for medium arousal pictures from pleasant to unpleasant valence. Note that this effect is only observed for 80 and 95, but not 105 dB probe stimulus intensity.

Figure 3. Response probability to low intensity probes across all valence levels for each arousal category. This graph illustrates the decrease in response probability from pleasant to unpleasant valence levels at all arousal categories. Also, low arousal pictures produce the most probable startle responses for each valence level. Medium arousal pictures are least probable to produce a startle response for neutral and unpleasant pictures.

Figure 1

Arousal effects across valence levels to medium intensity probes

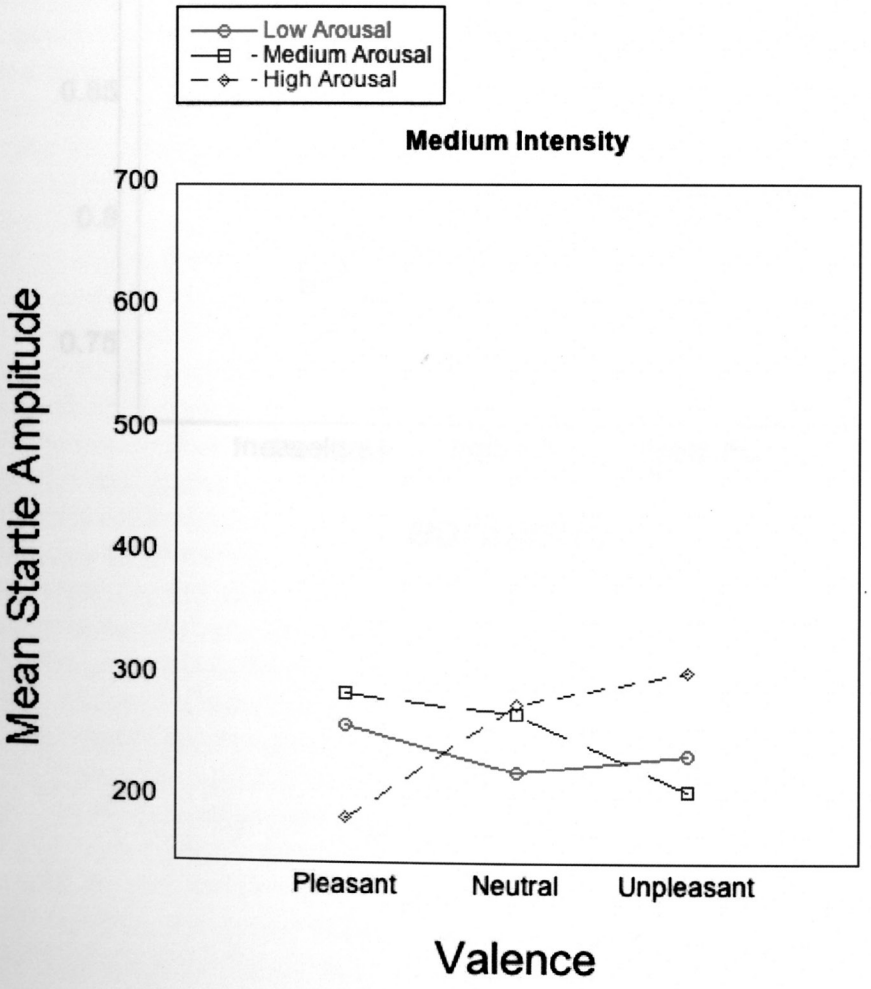

Figure 2

Medium arousal effects of valence at each probe intensity level

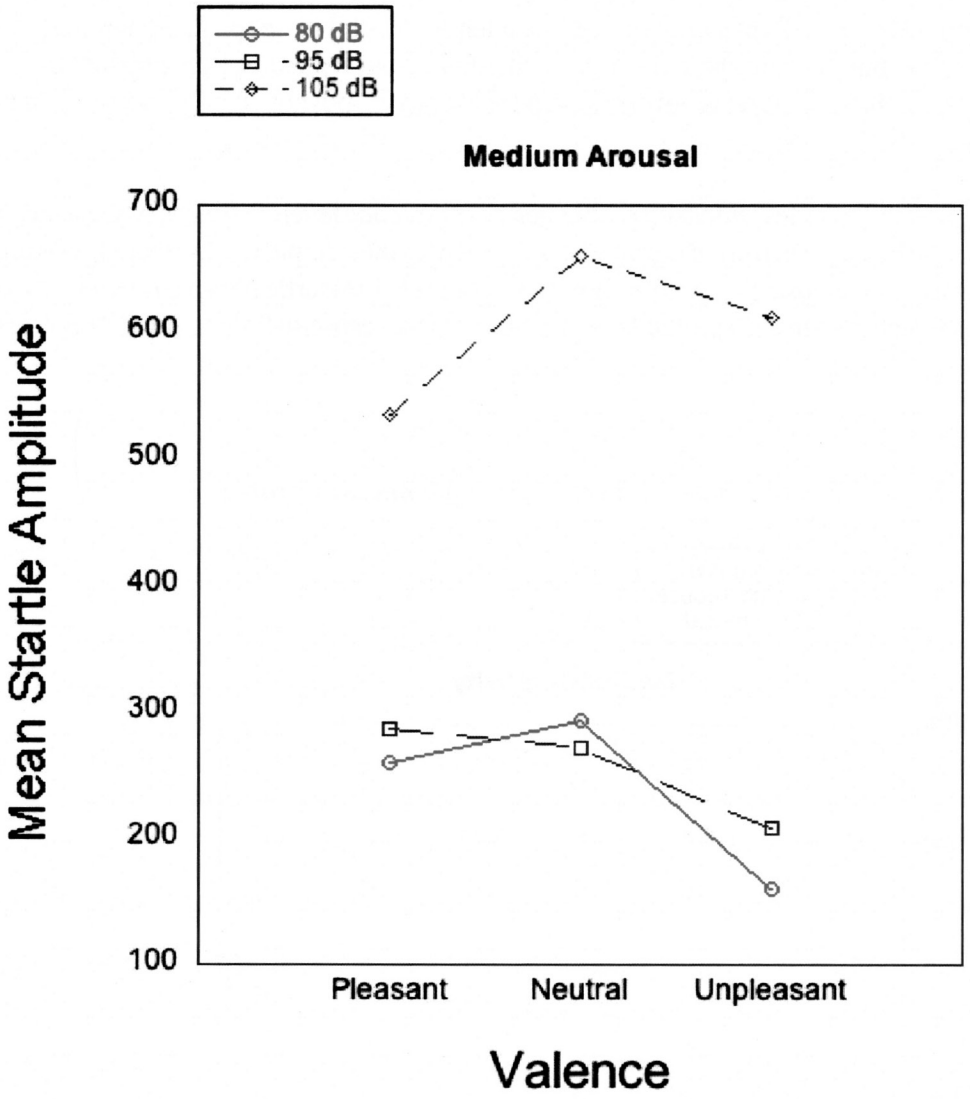

Figure 3

Response probability to low intensity probes across all valence levels for each arousal category