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Long term habituation and sensitization of the human acoustic startle response

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Abstract An experiment was conducted to explore the long-term effects of stimulus repetition on the human acoustic startle eyeblink response under different training interstimulus intervals (ISIs). Replicating and adapting a paradigm previously used with rats, two groups of human participants (n = 8) were presented with three successive days of training stimuli with ISIs of 2 s or 16 s. Identical pre- and posttests were given on days one and four, respectively. At posttest, significant long-term (across days) response habituation of blink probability was observed in the 16-s group but not the 2-s group; in contrast, a striking increment of blink amplitude was observed in the 2-s group. Experiment II verified that the training conditions employed in Experiment I could produce differential short-term (within session) habituation for 2 and 16-s ISIs.

Habituation and sensitization are described as two fundamental mechanisms of behavioral change observed in the context of repeated stimulation, the former associated with a decrement in responding, the latter with an increment. The dual-process theory of habituation (Groves & Thompson, 1970, 1973; Thompson, Groves, Teyler, & Roemer, 1973; see also Thompson, Berry, Rinaldi, & Berger, 1979) proposes that habituation occurs within specific stimulus-response pathways producing a decrement in responding while sensitization acts more generally, incrementing responding by producing a state change in the organism. As both mechanisms are proposed to be simultaneously active, the manifest change in behavior to a repeated stimulus (either decremental or incremental) represents a summation of their respective influences. This dual-process theory has served as the foundation (or foil) for much of the work regarding perceptual learning and stimulus repetition effects during the past quarter of a century (e.g., Davis & File, 1984; Graham, 1973; Hall, 1991).

In their classic definition of habituation, Thompson and Spencer (1966) provide nine parametric characteristics. Of these nine, three specify aspects of stimulus timing and presentation which can modify the magnitude and/or rate of habituation. Specifically, habituation may be facilitated by (a) low intensity stimuli, (b) previous training sessions with the stimulus, and (c) increasing the frequency with which the stimulus is presented. Support for these characteristics was provided using the flexion reflex of the spinal cat in which facilitated habituation was reported (a) following a recovery interval in a second series of habituation trials, and, (b) to stimuli presented once per second in comparison to once every 3.2 seconds (Thompson & Spencer, 1966). Additional support has been provided by experiments with the acoustic startle response in the rat (Davis, 1970a; see also Davis 1970b). During training on 1000 trials, rats presented with stimuli at 2 s intervals showed a greater decrement in responding than did the rats in a 16-s comparison group. However, when both groups were presented with identical test stimuli at multiple intervals, it was the 16-s-group that revealed greater response inhibition; this was true both when the test followed training after a 1 min interval and when testing was delayed for 24 hours, although the magnitude of the difference was nonsignificant at the 24-hour test. These results have been interpreted as indicating the existence of separable processes of short- and long-term habituation

(Davis, 1970 a; Davis & File, 1984; Petrinovich, 1984). By analogy, separable mechanisms of short- and long-term sensitization have also been proposed (Petrinovich, 1984).

For psychophysiologists, the startle response has a number of advantageous characteristics. Among these are (a) the ubiquity of the startle response or analogous startle-like rapid escape responses found across a range of organisms from fish and crustaceans to humans (Eaton, 1984; Landis & Hunt, 1939), (b) the identification of specific neural circuitry underlying the acoustic startle response in rats (Davis, Gendelman, Tischler, & Gendelman, 1982; Lee, Lopez, Meloni, & Davis, 1994), and (c) a readily quantifiable response in the human, that is, electromyographic measurement of the eyeblink reflex component of the startle response. Although, given the differences in absolute onset latency, questions have been raised about the degree of similarity between the neural circuitry for the acoustic eyeblink reflex in the human and that identified in the rat (Hackley & Boelhouwer, in press), the startle response nevertheless provides the opportunity of an investigative window on the neural substrates of a behavioral phenomenon observed in humans. For example, the work of Braff, Geyer, Swerdlow, and their colleagues on sensory gating and prepulse inhibition (e.g., Braff & Geyer, 1990; Kodsi & Swerdlow, 1995; Swerdlow, Braff, Masten, & Geyer, 1990; Swerdlow & Geyer, 1993) demonstrates, in the area of schizophrenia, the reciprocal benefit between human and rat studies using the startle reflex.

Habituation of the blink reflex or startle response in humans has been studied as a fundamental component of the basic response pattern in normal populations (Bradley, Lang, & Cuthbert, 1993; Lipp, Arnold, Siddle, & Dawson, 1994; Sanes & Ison, 1983) and in relation to a number of pathological conditions, such as Huntington's disease (Esteban & Giménez-Roldán, 1975), Parkinson's disease (Messina, Di Rosa, & Tomasello, 1972), and schizophrenia (Geyer & Braff, 1982). However, all of these investigations have examined short-term (within session) effects. There have been only two published investigations of long-term (across days) changes (Ornitz & Guthrie, 1989; Ornitz, Lane, Sugiyama, & de Traversay, 1993); both demonstrate a clear distinction between short-term and long-term effects and document the measurable existence of long-term effects in the human startle response in normal (Ornitz & Guthrie, 1989) and non-normal (i.e., autistic children; Ornitz et al., 1993) populations.*

As was indicated above, there appear to be separable mechanisms underlying short- and long-term habituation (Davis & File, 1984; Petrinovich, 1984) which have been demonstrated to be differentially sensitive to the interval between stimulus repetition during training in the rat acoustic startle reflex (Davis, 1970 a, 1970 b). However, an extension of these data to humans has not been previously reported.

The purpose of Experiment I was to investigate the long-term (i.e., across days) effects of stimulus repetition on the human acoustic startle eyeblink response under multiple training conditions. To do this, the procedure previously used with rats (Davis, 1970 a) was adapted for humans to explore the effect of training across three daily sessions with two different interstimulus intervals (ISIs, 2 s and 16 s). In order to assess the long-term effects of habituation training at each ISI, both groups of participants were also given multiple-ISI pretests and posttests including stimuli presented with 2-, 4-, 8-, and 16-s ISIs. Because these multiple-ISI tests were identical for both groups, the problems of confounded training and test ISIs inherent in habituation experiments such as this are diminished (Davis, 1970 a); moreover, because the posttest was conducted one day following the last sequence of training trials allowing sufficient time for any short-term (within-session) effects to dissipate, the results could be reasonably interpreted as the result of a long-term mechanism. It was expected that within each sequence of training trials, habituation would be facilitated by the increased stimulus presentation frequency (Thompson & Spencer, 1966)

* As a number of authors have mentioned (Peeke & Petrinovich, 1984; Thompson et al., 1973), during the past 25 years the terms *habituation* and *sensitization* have been used variously to refer to hypothetical processes resulting in specific behavioral change as well as to the behavior change itself. Although perhaps not an ideal solution, I will follow Thompson et al. (1973) and reserve the terms *habituation* and *sensitization* for the hypothetical processes and use *response habituation* or *response sensitization* to refer to the associated change in behavior.

such that response habituation would appear greater for the subjects receiving stimuli at 2-s ISIs as compared with the 16-s ISI group. It was also expected that the exposure to stimuli delivered at 16-s ISIs, repeated across multiple days, would produce increased long-term habituation as compared with the 2-s ISI condition (Davis, 1970a; Thompson et al., 1979). This long-term effect was expected to produce greater response habituation for the 16-s ISI group as compared with the 2-s ISI group at 24 h post test. This pattern of results would replicate and extend to humans those of Davis (1970a) with rats. Finally, it was of interest to examine the results from the current sample to see if the long-term (across-days) sensitization reported by Ornitz and Guthrie (1989) would be replicated, and if so what would be the effect of the different ISIs during training.

To foreshadow the results of Experiment I, a modest decrement in the probability of responding was observed in the 16-s group suggesting the expected effects of long-term habituation. This was overshadowed by the failure to observe the expected facilitation of within-session short term response habituation for the 2-s ISI group; rather, a robust facilitation of blink amplitude which continued across days was observed in the responses of the 2-s ISI group. A small second experiment was conducted which examined the within-session effects of the ISI manipulation in a single sequence of training trials without a pretest. This experiment verified that the facilitation of response habituation by increased stimulus presentation frequency, identified as a fundamental characteristic of habituation by Thompson and his colleagues (Thompson et al., 1979; Thompson & Spencer, 1966), could be observed, given the stimuli and measurement techniques employed in the first experiment.

All subject recruitment and testing procedures for these experiments were approved by the Universities' Institutional Review Boards.

Experiment I

Method

Subjects

Sixteen students (age range 18–24 years; 6 females) enrolled in an introductory psychology course at La Sierra University chose among several options to participate in this study and received course credit for their participation. The students were assigned to groups (n = 8) in a counterbalanced order during their initial visit to the laboratory, with the constraint that equal numbers of males and females were in each group. All students gave informed consent prior to their initial session of participation.

Apparatus

Reflex eyeblinks were elicited by 50 ms bursts of broadband white noise generated by a Coulbourn white noise generator (S41–02) gated through Coulbourn shaped rise/fall gates (S84–04) set to 5 ms rise/fall times. The noise was amplified by a Pioneer SX-2600 amplifier and presented binaurally to participants via TDH-50 earphones with hard rubber, supra-aural cushions. Noise levels were calibrated using a Quest (Model 2700) sound level meter and appropriate coupler to 95 dB(A) at each ear.

Data acquisition and the timing of stimulus presentation were controlled by a 386-based PC-compatible computer using Contact Precision Instruments (CPI) PSYLAB software (1991). The electromyogram (EMG) was recorded from the orbicularis oculi muscle using miniature Sensor Medics Ag/AgCl electrodes taped on the lower lid of the left eye (Fridlund & Cacioppo, 1986). EMG was amplified and bandpass filtered (CPI Bioamplifier, SN9126, 0.1–1000 Hz) before being rectified, integrated (CPI Rectifier/Integrator, SN9109, Time Constant = 100 ms) and digitized online by a CPI MC-24 A/D converter (SN9075) sampling at 1000 Hz with 12-bit resolution. The EMG signal, beginning 25 ms prior to the onset of the noise bursts and continuing for a total 250 ms, was stored on disk for offline analysis.

Procedure

Students participated in four experimental sessions, returning to the laboratory at the same time on four successive days. All appointments were at noon or 5 p.m., on Mondays through Thursdays. On Monday, after obtaining informed consent, electrodes were placed, subjects were seated in a comfortable chair in the testing room and put on the headphones. Subjects were informed that they would hear a

number of loud sounds spaced at irregular intervals. They were instructed to remain awake and alert and to refrain from unnecessary movement.

At this point, all subjects received an 81-stimulus pretest which consisted of an initial stimulus plus 20 trials at each of four ISIs (2, 4, 8, and 16 seconds, measured from stimulus onset to stimulus onset). Following the initial stimulus, the 80 trials were arranged in 4 blocks of 20 trials with a pseudorandom sequence of ISIs such that each block contained five trials at each ISI and that a given ISI never occurred on more than two consecutive trials. All 81 trials of the pretest were presented in a single continuous sequence. After the pretest, subjects were informed that they would next hear another series of stimuli but that these would occur at more regular intervals. This sequence of training trials consisted of an initial stimulus and 56 stimuli presented at a constant (2- or 16-s) ISI.

Subjects returned to the laboratory on each of the two subsequent days (Tuesday and Wednesday) and, after having the electrodes re-applied, were presented each day with 85 stimuli (an initial stimulus plus 84 stimuli) at the constant ISI. On Thursday, all subjects were given the 81-stimulus posttest; the posttest was identical to the pretest except that ISIs occurred in a different pseudorandom order. After the posttest, subjects were debriefed, given an opportunity to ask questions, and thanked for their participation.

Data analysis

Eyeblink EMG were scored offline for amplitude, onset latency, and probability. A custom, automatic scoring program (BlinkScore v1.1, Haerich, 1995) searched each trial for blink response onset defined as the point, within a window of 20–140 ms after stimulus onset, at which the EMG began a positive deviation of at least 40 a/d units within 3 ms. Peak amplitude was scored relative to the pre-response baseline as the maximum deviation occurring within 220 ms of stimulus onset. The mean EMG level for the 20 ms prior to the point of onset was considered as the baseline for calculating amplitude. If no blink onset could be determined the trial was considered a "No Blink" trial for purposes of calculating blink probability and was excluded from calculations of latency or amplitude means. Trials in which EMG activity occurring before stimulus onset or within 20 ms of onset made determining a valid blink onset impossible were classified as "Unscorable" and omitted from all analyses (less than 4% of all trials). Blink probability was calculated as the ratio of blink trials to the total number of scorable trials.

Means for each measure (amplitude, latency, and probability) were calculated across blocks of 7 trials, omitting the initial trial, for each of the sequences of training trials (on Monday, Tuesday, and Wednesday). Similarly, means were calculated for each of the ISI conditions in the pretest and posttest. In all cases, calculation of these means was based on the remaining trials after unscorable trials were omitted. These means were used in statistical analyses. The initial trial for each of the two test sequences was considered separately. Greenhouse-Geisser corrected *P*-values are reported along with uncorrected degrees of freedom in cases of possible violation of the sphericity assumption in analyses of variance.

Results

Pre- and posttests

Inspection of the top panel of Figure 1 which presents the data for the pretests and posttests indicates that, while the two groups were similar to each other on all measures in the pretest, by posttest a striking increase in blink amplitude occurred for the 2-s ISI group. This was confirmed in the analysis of variance (ANOVA) as a significant TEST × GROUP interaction ($F(1,14) = 6.85$, $P < .05$). Subsequent planned pairwise comparisons indicated an increase in amplitude at each ISI for the 2-s group (all ts $(7) > 3.74$, Ps $< .05$), but no reliable change in amplitude for the 16-s group (ts $(7) < 1.0$). In the blink probability measure, ANOVA revealed a significant three-way interaction between TEST DAY, ISI, and GROUP ($F(3,42) = 5.43$, $P < .05$). Planned pairwise comparisons indicated that significant decreases in probability occurred from pretest to posttest for the 16-s group at 4, 8, and 16-s ISIs (ts$(7) > 2.66$, Ps $< .05$), but not at the 2-s ISI; no significant changes were found for the 2-s group. Blink latencies ranged from 46 to 63 ms; however analyses of this measure revealed no reliable main effects or interactions.

The pretest and posttest each consisted of 81

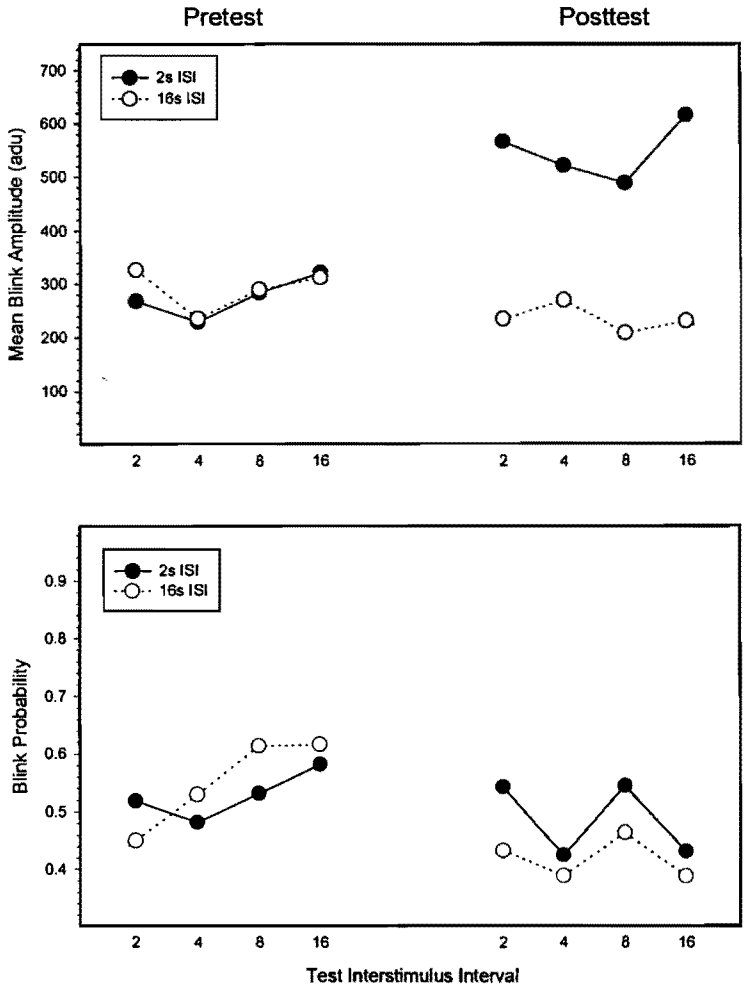

Figure 1 Mean blink amplitude (top panel) and probability (bottom panel) for the pretest and posttest of Experiment I. The pretest and posttest were identical and were presented on the first and fourth day, respectively. Adu are arbitrary analog/digital units.

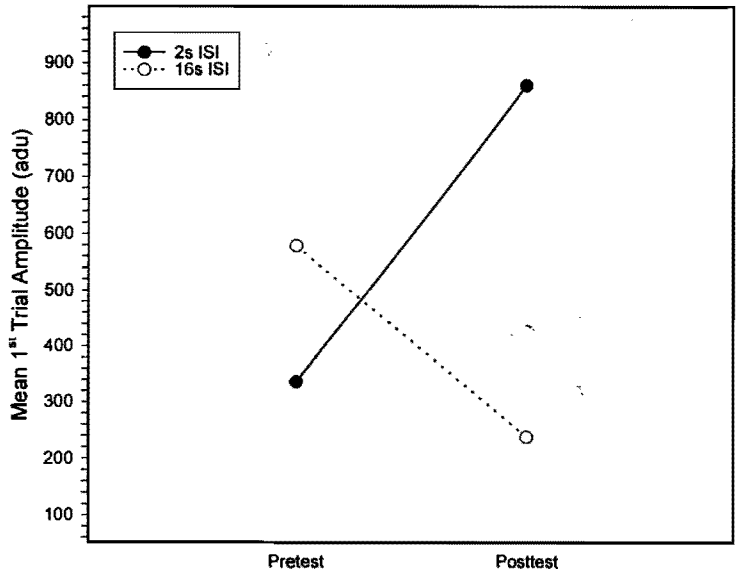

Figure 2 Mean blink amplitude to the first stimulus of the pretest and posttest of Experiment I. Adu are arbitrary analog/digital units.

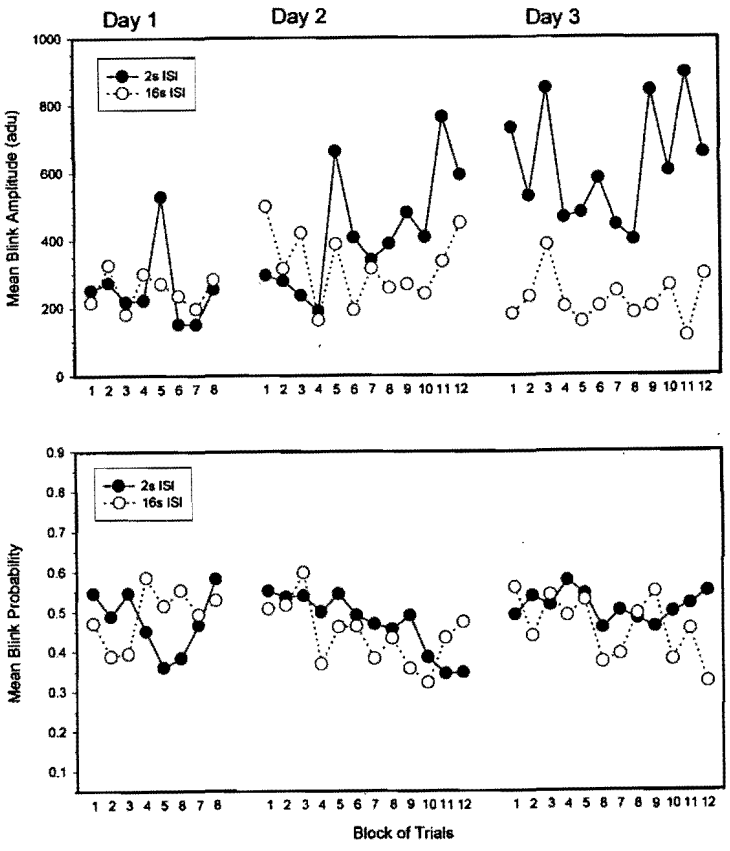

Figure 3 Mean blink amplitude (top panel) and probability (bottom panel) across the three daily sequences of training trials in Experiment I. Seven trials were presented for each block. Adu are arbitrary analog/digital units.

stimuli. Blink amplitudes and latencies elicited by the initial stimulus from each test day were analyzed separately. The probability measure was not included as all subjects produced responses to the initial stimulus. As may be observed in Figure 2, the mean blink amplitude to the initial stimulus increased from pretest to posttest for the 2-s group but decreased for the 16-s group, (TEST × GROUP interaction: $F(1, 14) = 22.18$, $P < .001$). A complementary pattern of change may be observed in the blink latency measure; however, the TEST × GROUP interaction was not reliable ($F(1, 14) = 3.23$, $P = .09$).

Since changes in baseline, pre-response muscle activity might provide a mediating explanatory mechanism for the enhancement of amplitude observed in the 2-s group at posttest, an analysis of variance was conducted on the baseline EMG. This analysis revealed no reliable main effects or interactions.

Training trials

The increase in blink amplitude apparent in the posttest data for the 2-s ISI group may be observed to develop across the sequences of training trials (Figure 3, top panel). Analyses of variance (BMDP2V with orthogonal trends) revealed a significant linear trend across trial blocks for the 2-s group ($F(1, 7) = 6.04$, $P < .05$) as well as a LINEAR TREND × GROUP interaction ($F(1, 14) = 7.87$, $P < .05$); the linear trend was not significant for the 16-s group, nor were any other effects in the analyses of amplitude. Similar analyses for blink probability (Figure 3, bottom panel) and latency revealed no significant main effects, linear trends, or interactions.

Visual inspection of the bottom panel of Figure 3 suggests that short-term or within-session habituation may have occurred, particularly for the 16-s ISI group. Since the training trials in the first training session were preceded by the pretest and included only 57 stimuli, whereas the second and third day's sessions consisted only of the 85 training-trial stimuli, separate analyses of probability and latency data for the second and third days were conducted. The analyses of probability revealed significant linear trends across blocks for each

day (Fs(1,14) > 15.05, Ps < .01), which did not interact with the group factor. The three-way interaction with DAY, however, was significant, (LINEAR TREND × DAY × GROUP: $F(1,14) = 6.70$, $P < .025$). Additional ANOVAs for each group were conducted to explore this three-way interaction. For the 2-s ISI group, both the LINEAR TREND and the LINEAR TREND × DAY interaction were significant (Fs(1,7) > 5.60, Ps < .05), while for the 16-s ISI group only the LINEAR TREND across blocks was significant ($F(1,7) = 12.52$, $P < .01$; interaction, $F(1,7) = 1.06$). These results suggest similar within-session decreases of reflex probability for the 16-s ISI group across both days' training trials. For the 2-s ISI group, response habituation of probability was greater on the second day than on the third day. Once again, analyses of latency data revealed no reliable effects, trends, or interactions.

Discussion

The most salient result from this experiment was the increase in blink amplitude which occurred between pretest and posttest for subjects in the 2-s ISI group. This response sensitization may be observed to develop across the three sequences of training trials. Moreover, it was observed in the posttest (including the initial trial) which occurred 24 hours after the third day's training trials. Taken together, these observations suggest the occurrence of a robust long-term response sensitization of the amplitude of the blink reflex.

The results from Experiment I may be considered, on the one hand, to differ in two respects from what was expected based upon the previous data collected with rats (Davis, 1970a). First, in that study, response habituation was observed across the training trials for both groups with the 2-s ISI group of rats responding with a lower probability than the 16-s ISI group. In the current study, response habituation was observed within sessions in the blink probability measure; however, the only reliable effect of ISI was a decrease in the within-session response habituation for probability in the 2-s ISI group, the opposite of the within-session effect reported by Davis. Second, both groups of rats showed habituation in the posttest with greater habituation observed in the rats trained with a 16-s ISI. This response habituation was observed with posttests conducted both immediately and with a 24-hour delay (although post-hoc testing indicated that the effect on the delayed posttest was of marginal reliability). In the current experiment, only the humans in the 16-s ISI group showed habituation of the blink reflex at the posttest. In contrast, response sensitization (of blink amplitude) was observed in subjects in the 2-s ISI group; Davis (1970a) reported no response sensitization.

On the other hand, it is possible to emphasize certain similarities between the two studies. For example, when restricted to the dependent measure used by Davis (1970a), startle probability (rather than response amplitude), it is found that the 16-s ISI groups, both rats and humans, exhibited greater response habituation at the posttest than did their respective 2-s ISI cohorts.

The short term (within-session) response habituation observed in the second and third sequences of training trials of the current experiment appeared to be decreased by the shorter interstimulus interval, contrary to expectation. Given this result, it seemed prudent to ascertain whether or not the facilitation of response habituation by increased stimulus presentation frequency, identified as a fundamental characteristic of habituation by Thompson and his colleagues (Groves & Thompson, 1970; Thompson et al., 1973, 1979; Thompson & Spencer, 1966), and which has been repeatedly documented in multiple response systems including the human reflex blink (Boelhouwer, Gregoric, van den Bosch, Schomaker, & Brunia, 1982; Davis, 1970a; Gatchel, 1975; Thompson et al., 1973; Thompson & Spencer, 1966), could be observed, given the stimuli and measurement techniques employed in the first experiment.

Experiment II

The purpose of this second, small experiment was to verify that the ISI manipulation used in the first experiments could produce differential habituation within a single sequence of training trials. No multiple-ISI pre- or posttests were run; and all training trials were presented in a single sequence on one day. It was expected that the probability of a reflex blink would be less for subjects receiving stimuli every 2 s

in comparison with subjects receiving stimuli every 16 s. Because the facilitated within-session habituation produced by increasing the frequency of training-stimulus presentation is presumably a robust phenomenon, it was additionally expected that the effect would be observable with a small number of subjects.

Method

Subjects

Nine students (age range 18–20 years) who had not taken part in the previous experiment participated for credit in an introductory psychology course. Five subjects (2 females) were assigned to the 2-s ISI group, four subjects (2 females) to the 16-s group.

Apparatus and procedure

Stimulus presentation and data acquisition were identical to Experiment I. The procedure was identical except that 141 stimuli (an initial stimulus plus 140 training stimuli) were presented with either a 2-s or 16-s ISI in a single session on one day. There was no pretest or posttest in Experiment II.

Results and discussion

The data for blink amplitude and probability are presented in Figure 4. As expected, greater response habituation of the acoustic startle was observed with increasing frequency of stimulus presentation. Specifically, the probability of a response declined for both groups across blocks of trials (linear trend: $F(1,7) = 6.81$, $P < .05$). There was a greater decrement in responding for the 2-s ISI group than for the 16-s group as is suggested by the GROUP × LINEAR TREND across BLOCKS interaction ($F(1,7) = 10.72$, $P < .01$), and the marginally reliable main effect of GROUP ($F(1,7) = 4.43$, $P = .07$). Thus, the results of the current experiment with human participants parallel those reported by Davis (1970a) with rats.

None of the effects or interactions in the analysis of blink amplitude reached significance. However, four of the trial blocks in the data for the 2-s ISI group display extraordinarily large mean amplitudes in comparison to the surrounding blocks. These large means resulted from the contribution of a single subject. When analyses were conducted omitting that

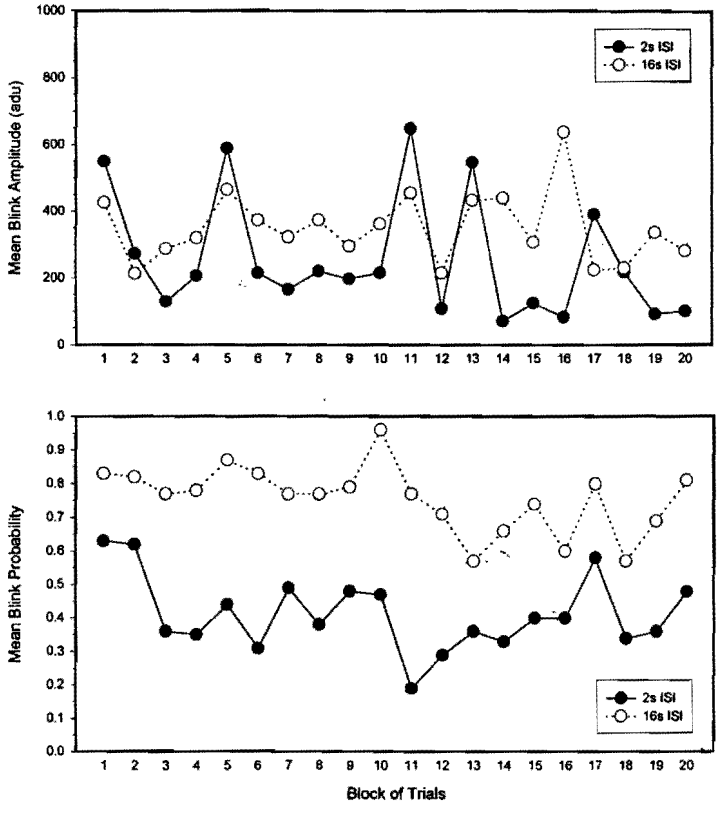

Figure 4 Mean blink amplitude (top panel) and probability (bottom panel) across blocks of training trials in Experiment II. Seven trials were presented for each block. Adu are arbitrary analog/digital units.

subject's data, the LINEAR TREND across BLOCKS × GROUP interaction approached significance ($P = .08$). These results imply that the facilitatory effect of more frequent stimulus presentation on response habituation was obtainable with the stimuli and measurement techniques employed in Experiment I, but that the size of the effect was not as great as was expected.

General discussion

The primary purpose in undertaking Experiment I was to investigate the long-term (across-days) effects on the human acoustic startle response of varying the interstimulus interval. The experimental paradigm employed was based on work with rats (Davis, 1970 a), the intent being to extend the area of cross-species comparison available with startle methodologies. The results of Experiment I indicated that, as expected, the human participants performed similarly to the rats in that there was a greater response decrement, measured as blink probability at posttest, in the group which received training stimuli at 16-s intervals as compared with a group receiving 2-s ISI training. The response decrement was observed 24 hours after the last sequence of training trials, an interval over which any short-term habituation would presumably have dissipated. Furthermore, a decrement in blink amplitude was observed on the very first trial of the test session prior to the re-development of any short term habituatory mechanisms. Taken together, these observations suggest that long-term habituation of the acoustic startle is similarly responsive in humans as in rats to the interstimulus interval employed in training.

Although the habituation of response probability for the 16-s group was statistically reliable at test ISIs of 4, 8, and 16 s, the magnitude of the habituation cannot be called large. This may be regarded as unsurprising given the relatively small amount of training. Participants in Experiment I received 223 training trials distributed across three days in contrast to the rats in Davis' experiment (1970 a) that received 1000 training trials in a single session. The current data may be speculatively placed within the context of previous work with humans by emphasizing the effect of increasing the training ISI on the production of long-term habituation. Specifically, Ornitz and Guthrie (1989) demonstrated long-term habituation in the human acoustic startle response using a 23 s ISI. Experiment I in the current study showed a small but reliable habituation effect using a 16-s ISI, but no effect of training with a 2-s ISI. Thus, it may be that the range of ISIs necessary for the induction of long-term acoustic startle habituation may have a lower bound near 10–16 s and extend with increasing effect to 23 s and beyond.

However, the preceding account ignores the most striking result in Experiment I, a conspicuous potentiation of blink amplitude observed from pretest to posttest in the 2-s ISI group. It is unlikely that this response sensitization was due to short-term or transient effects of stimulus repetition since it was observed 24 hours after the last sequence of training trials and was evident on the first trial of the posttest. Furthermore, its development may be observed in the blink amplitude data from the training trials.

While this effect was unexpected in the current study which was patterned after Davis' (1970 a) study, and in need of replication, it may not be totally without precedent. In another experiment conducted with rats which used shorter training sessions (50 vs. 1000 stimuli) and measured response amplitude rather than response probability (Davis, 1972), sensitization was observed. In that experiment, rats received two training sessions per week for four weeks. Startle amplitude habituated during the initial session but increased during each subsequent session, producing a pattern of results similar to that observed in Experiment I.

In addition, it should be acknowledged that receiving a 95 dB noise burst every two seconds is likely to be an aversive rather than an appetitive experience for most people. As such, the laboratory context (the testing room, application of electrodes, etc.) may have served as an aversive conditioned stimulus effectively evoking a negative affective state by the third day of training and, *a fortiori*, on day four during the posttest. Fear-potentiation of the acoustic startle response has been extensively studied in both rats (Davis, in press; Davis, Hitchcock, & Rosen, 1987) and humans (Hamm & Vaitl, 1993; Lang, 1995; Lang, Bradley, & Cuthbert, 1990). It seems reasonable to expect that, if such a conditional effect did occur, it would be

manifested in an increase in blink amplitude. Indeed, Leaton and his colleagues have reported that fear conditioning may occur in conjunction with startle habituation testing in rats (Borszcz, Cranney, & Leaton, 1989, Young & Leaton, 1994). Furthermore, the central nucleus of the amygdala which mediates the conditioned fear-potentiation of acoustic startle (Hitchcock & Davis, 1986) also appears to mediate the facilitatory effects of "habituation testing" as lesions of this nucleus reduce both the freezing and cardiac responses associated with startle facilitation (Young & Leaton, 1996).

The current experiments were conducted to explore the effects on the human acoustic startle response of stimulus repetition at differing ISIs. The parametric characteristics of habituation (Thompson & Spencer, 1966) predict that increasing the frequency of stimulus presentation and providing sessions of training trials across multiple days should facilitate habituation of the response. The results of Experiment II are consonant, albeit marginally, with this prediction; both response probability and response amplitude were lower with the more frequent stimulus presentation condition. In Experiment I, more frequent stimulus presentation did not produce an observable increase in response habituation on any of the four days; neither did multiple days of training result in an observable progressive facilitation of response habituation.

One explanation for the relative lack of response habituation might be found in the way humans respond to a rather boring situation.* In an effort to comply with the experimenter's instructions to remain awake and alert, but refrain from unnecessary movement, a subject might employ various strategies which alter attentional focus and level of arousal. Such strategies would likely have an activating effect on motoneurons, counteracting any habituatory mechanisms. The analysis of baseline EMG between pretest and posttest argue against this as a sufficient explanation for the increase in response amplitude observed in the 2-s ISI group. Similarly, the absence of changes in baseline EMG might be regarded as evidence that the expected habituation of neuronal activity did not occur and an attention-based source of activation cannot be ruled out.

The putatively co-active mechanism of sensitization provides another perspective. Because sensitization is defined as producing an effect which is antagonistic to and additive with habituation (Groves & Thompson, 1970, 1973; Thompson et al., 1973), it is difficult to say whether an absence of increasing response decrement across days reflects a lack of long-term habituation or an off-setting amount of long-term sensitization. Furthermore, sensitization, like habituation, is often considered to represent perceptual rather than associative learning (Hall, 1991). Thus, if the increase in blink amplitude observed in Experiment I reflects a conditional facilitation resulting from the aversiveness of the 2-s ISI condition, one may legitimately question whether the effect may even be properly described as long-term sensitization. To the extent that the results of Experiment I parallel the phenomena described by Leaton and his colleagues (Borszcz et al., 1989; Young & Leaton, 1994, 1996), the answer would be that it is not sensitization. Additional information might be provided by assessing multiple response pathways (i.e., other reflexes) under different test conditions. Sensitization, as a systemic or general mechanism of potentiation would be expected to be relatively insensitive to a change in context and would produce facilitation of responses across a variety of stimuli.

In conclusion, the results of the current two experiments, in which effects of stimulus repetition at two different ISIs were investigated, considered together with those of Ornitz (Ornitz et al., 1993; Ornitz & Guthrie, 1989) and in comparison with data from rats (Borszcz et al., 1989; Davis, in press; Davis & File, 1984; Davis et al., 1987; Young & Leaton, 1994, 1996) suggest the utility of continued investigations into the long-term effects of stimulus repetition on the human startle response.

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