

# Habituation of the eyeblink response in humans with stimuli presented in a sequence of incremental intensity

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## ABSTRACT

In an experiment we examined whether the repeated presentation of tones of gradually increasing intensities produces greater decrement in the eyeblink reflex response in humans than the repetition of tones of constant intensities. Two groups of participants matched for their initial level of response were exposed to 110 tones of 100-ms duration. For the participants in the incremental group, the tones increased from 60- to 90- dB in 3-dB steps, whereas participants in the constant group received the tones at a fixed 90-dB intensity. The results indicated that the level of response in the last block of 10 trials, in which both groups received 90-dB tones, was significantly lower in the incremental group than in the constant group.

These findings support the data presented by Davis and Wagner (7) with the acoustic response in rats, but differ from several reports with autonomic responses in humans, where the advantage of the incremental condition has not been observed unambiguously. The discussion analyzes theoretical approaches to this phenomenon and the possible involvement of separate neural circuits.

**Key words:** habituation, incremental stimulus intensity effect, sensitization.

## INTRODUCTION

When a stimulus is systematically repeated, the predominant result is a progressive diminution in the frequency or amplitude of the response. When it is proved that this decrement is prolonged over time and that it is not caused by either muscular fatigue or sensorial adaptation, it is inferred that a learning phenomenon known as habituation has occurred (14, 27).

The universality of this simple form of learning has been demonstrated in a broad range of organisms such as protozoa (33), birds (8), fish (22), mollusks (11), rats (3), rabbits (32), cats (13, 27), dogs (23) and humans (9), just to name a few. A good deal of research has focused on determining the conditions or parameters that lead to habituation (26, 27). Although it seems to be a well-established fact that the effectiveness of habituation depends on the number and spacing of the repetitions, the experimental evidence is less clear regarding other factors, such as the intensity of the stimulus.

In this last category, there is a phenomenon known as "incremental stimulus intensity effect" (ISIE), which refers to the observation that habituation is more effective when the repetition of the stimulus involves progressive increments in its intensity than when the intensity is constant (13). The existence of this effect has been taken as evidence favoring the so-called Dual-Process Theories of Habituation (e.g., 13), which argues that in addition to the decremental tendencies that are specific to the stimulus in question (habituation), the repetition of the stimulus also produces generalized decrements in the form of loss of arousal or desensitization, the latter explaining ISIE.

The first systematic observations of ISIE were made in experiments on instrumental conditioning, in which animals were trained to produce an operant response rewarded with food, which was presented simultaneously with an electric shock (1, 16, 18). The results indicated that even though the punishment provoked a suppressing effect over the rewarded behavior, this effect progressively disappeared over the trials,

and that this decrement was stronger when the punishment was delivered with shocks of incremental rather than constant intensities. Despite the suggestive character of these findings, it is not clear whether the decrement in "aversiveness" to the electric shock was due to habituation, since this might also be interpreted as the development of antagonistic behavior that aided the animals to avoid the shocks, or as the formation of an association between the shock and the reward (7).

These difficulties led to studies in which the stimulus in question was repeated under conditions in which there were neither rewards nor obvious possibilities to avoid the aversive stimulus by expressing certain behaviors. The first of these studies was conducted by Church, LoLordo, Overmier, Solomon and Turner (2), who demonstrated that habituation to a cardiac acceleration response provoked by electric shock was greater in a group of dogs that received shocks of increasing intensities (from 0.5- to 6- mA) than another group that received the stimuli at a fixed intensity. Davis and Wagner (7, Experiment 2) studied the same effect in rats by comparing the acoustic startle response to 120-dB tones in groups of rats that had been exposed to 750 stimuli at either a constant 120-dB, a constant 100-dB, a random order of intensities between 85 and 120-dB, or a gradually increasing order of intensities between 85 and 120-dB. The findings indicated that there was substantially less response in the test with a 120-dB tone in the group that had experienced gradually increasing intensities than in any of the other three groups. Groves and Thompson (13, Experiment 3) essentially replicated these findings with the limb flexion reflex in the spinal cat.

In contrast to studies with dogs, rats and cats, the evidence in humans is not very clear. For example, O'Gorman and Jamieson (20, 21) demonstrated that the progressive presentation of an acoustic stimulus (between 80- and 100-dB, Experiment 1, 20; between 64- and 100-dB, 21) caused a higher decrement in electrodermal response than did constant presentations (100-dB). However, such an effect was

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not replicated when they measured the finger blood volume response (20, Experiment 1) and the cardiac response (20, Experiment 2). Similar difficulties to find this effect have been reported in procedures that employed galvanic skin and cardiac responses to phobic images (12) and electrodermal response to acoustic stimuli (17).

The absence of robust evidence of ISIE in humans casts doubts on its generality. Facing this ambiguity of results, it is necessary to take a look at the differences and similarities between studies that have found the effect and those that have not. On the one hand, most of the positive results have been obtained by examining skeletal responses in animals, such as the startle response in rats (7) and the limb flexion reflex in the spinal cat (13), which are typically of slow habituation. On the other hand, studies that have tested ISIE in autonomic responses, such as electrodermal and cardiac responses, which are both of more rapid habituation, have only demonstrated ISIE when the constant group has not shown detectable habituation (2, 20, 21). In contrast, when the habituation has been achieved in the constant group, the effect tends to disappear (17), possibly due to a floor effect that could complicate the detection of differences between the constant and incremental procedures. Thus, it could be argued that the ISIE can only be detected when the response is of slow habituation (skeletal) or when it is tested in the early stages in the development of habituation of autonomic responses. Of course, given the limited number of studies in this area, this is only speculation.

Taking into consideration the theoretical and empirical importance of this phenomenon, the methodological difficulties to observe pre-asymptotic habituation of autonomic responses and the absence of studies with human skeletal responses, this investigation examined the ISIE with a skeletal response typically used in studies of human habituation, eyeblink response. The habituation of this response has been relatively well studied in humans and has the advantage of requiring a considerable number of trials to reach asymptotic levels of habituation (9).

## METHOD

### *Participants*

A total of 72 male and female undergraduate students of the University of Talca, with a mean age of 18.2 years ( $SD = 0.23$ ), participated in the experiment for course credit. They were tested individually and had no previous experience in similar research.

### *Apparatus*

The experiment was carried out in a dimly illuminated (18 w bulb) and acoustically isolated room (2.5 m x 2.7 m x 2.4 m). The presentation of the stimulus and the recording of responses were controlled by the Eyeblink Conditioning System (San Diego Instruments, San Diego, CA), which administered the acoustic stimulus and registered eyeblink responses. The acoustic stimulus was a 100-ms tone, presented through MAICO earphones.

The eyeblink response was measured by a low power infrared photoelectric emitter/receiver that measures the amount of light reflected as the eyelid closes. The changes in

reflected light as blinks occurred were converted to changes in electrical signals that were analyzed by a computer program. The photoelectric cell was located in front of the participant's right eye and was supported by a headband to keep it in a fixed position throughout the experiment.

### *Procedure*

The experiment consisted of 4 phases: adaptation, pretest, habituation and post-test. In the adaptation phase, the researcher placed the headband with the stimulation and registration devices on the participant's head and calibrated its position to obtain a detectable eyeblink response. The experimenter then left the room and allowed the participants to adapt to the situation for 3 minutes without stimulation. In the pretest phase the participants received 5 tones of 90-dB at 40 sec intervals. The objective of the pretest was to determine the average level of response to the 90-dB tone before habituation. In the habituation phase, the participants were exposed to 100 presentations of a tone at 40-sec intervals. The constant group received these habituation sequences in a constant intensity of 90-dB, whereas the incremental group began with 10 tones of 60-dB rising gradually by 3-dB to the successive blocks of 10 sequences, until reaching a maximum of 87-dB. Finally, during the post-test phase, the participants in both groups received 10 trials of 90-dB tones.

### *Scoring*

Movements of the participant's eyelid were recorded with a frequency of one sample every 1-ms, which were expressed as changes in the voltage transmitted by the transducer. A standardization trial was conducted with one naive volunteer to obtain a measure of the amplitude of a typical eyelid response to the tone. The maximal voltage obtained during the 200-ms following the onset of the stimulus was regarded as a response amplitude of 100. The responses of all participants were expressed as a percentage of this standardization value.

The measure of the evoked responses was based on the maximal amplitude occurring within the 200-ms following the onset of the stimulus. An eyelid response was scored only if the record indicated an amplitude of 5% or more within the 100-ms of stimulus duration. A valid trial was defined as one in which the amplitude of response was lower than 5% within the 200-ms window that preceded the onset of the stimulus.

### *Results*

Figure 1 shows the mean amplitude of response of the two groups during the pretest with the 90-dB tones, over blocks of 10 trials when the groups received different intensities of the tones, and on the post-test in which each group was exposed again to the 90-dB tones. First, it is observed that both groups experienced a considerable decrement in responding to the 90-dB tone from pretest to post-test, indicating that the 100 habituation trials were effective in producing habituation in both conditions. Second, it can be seen that although the two groups exhibited similar amplitudes of responses during the pretest, there was considerably more response in the constant than in the incremental group in the post-test. This greater decrease in response in the incremental group supports

the idea that the incremental sequence is more efficient in producing habituation than the constant sequence.

The reliability of these observations was confirmed by a 2 (test: pretest, posttest)  $\times$  2 (group: constant, incremental) mixed design ANOVA. In order to avoid a loss of statistical power if the amplitude of response in the post-test were more uniform than in the pretest, a blocking factor of 2 levels based on the participants' initial amplitude of response was introduced (19). The blocking factor was obtained by dividing the participants into two groups using the median amplitude of response in the pretest as a cut-off point.

The ANOVA showed a significant main effect of test ( $F(1, 38) = 186.363$ ;  $p < 0.001$ ;  $\eta^2$  partial = 0.831) and blocking ( $F(1, 38) = 69.332$ ;  $p < 0.001$ ;  $\eta^2$  partial = 0.646), and no reliable main effect of the group ( $F(1, 38) = 0.664$ ;  $p = 0.420$ ;  $\eta^2$  partial = 0.017). There were also reliable interactions between test and group ( $F(1, 38) = 6.376$ ;  $p = 0.016$ ;  $\eta^2$  partial = 0.144) and between test and blocking ( $F(1, 38) = 56.641$ ;  $p < 0.001$ ;  $\eta^2$  partial = 0.598). The interactions between group and blocking and between group, blocking and test were non-reliable ( $ps > 0.901$ ).

All the effects related to the blocking factor confirm the utility of this procedure, especially the interaction between blocking and test, since it reflects the fact that the differences between the high and low responders tend to disappear in the post-test. The most interesting effects are the main effect of test and the interaction between group and test. On the one hand, the effect of the test reveals the existence of a decrement in both groups from pretest to post-test, which confirms the effectiveness of both procedures in producing habituation. On the other hand, the interaction between group and test was assessed by evaluating the simple effects of group in each test. This analysis indicated that the two groups showed no differences between them in the pretest ( $p = 0.339$ ), but did differ in the post-test, where the incremental group responded significantly less than the constant group ( $p = 0.037$ ).

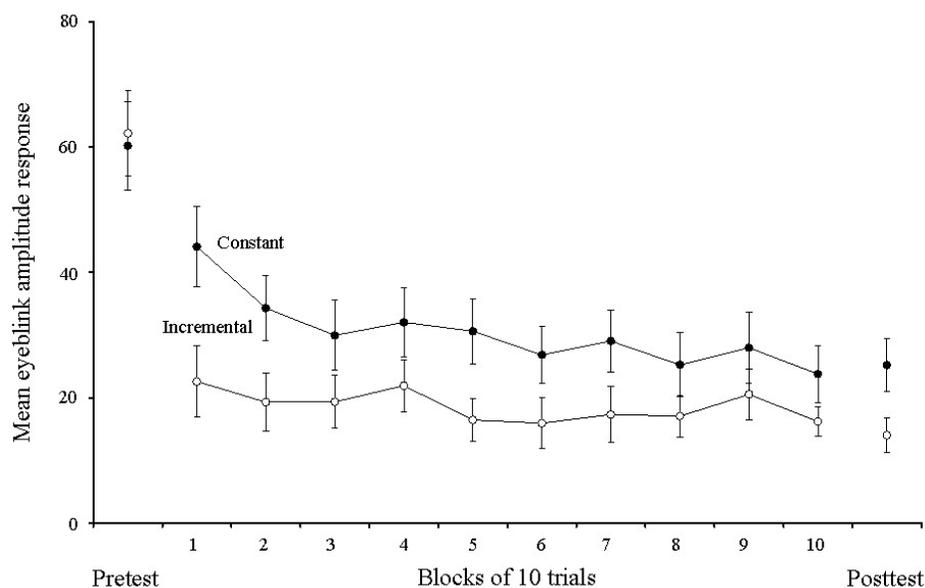
An interesting aspect of the data shown in Figure 1 is the demonstration of the incremental intensity effect, even though substantial evidence of habituation in both groups was obtained. This is contrary to the observations in which the incremental effect appears only when habituation had not yet occurred in the constant group (2, 20). In addition, Figure 1 shows extra evidence of habituation in both groups in that the data indicated a progressive decrement in the amplitude of the response within the 10 blocks of the habituation phase. Naturally, this drop is less marked in the incremental group since the decremental tendencies compete with the progressive increase in the stimulus intensity, although in the end, the decrease tends to predominate.

## DISCUSSION

The results of this investigation provide positive evidence of the existence of the incremental stimulus intensity effect in the habituation of the eyeblink response in humans. This information represents the first demonstration of this effect with skeletal responses in humans and is in agreement with the studies reported by Davis and Wagner (7) and Groves and Thompson (13) on the startle response in rats and the limb flexion reflex in the spinal cat, respectively.

As mentioned above, even though there is some evidence of the ISIE in the habituation of the autonomic responses, such as the cardiac response in dogs (2) and the electrodermal response in humans (20, 21), there is also evidence of null effects (with the electrodermal response, 17, and with the blood volume response, 20). Remarkably, the positive effects seen in the literature tend to match with poor habituation in the constant group.

It could be inferred that what produces controversial results is the quickness of the habituation of autonomic responses. If this were the case, the absence of the effect



**Figure 1.** Mean amplitude of eyeblink response of the constant group (black dots,  $n=36$ ) and incremental group (white dots;  $n=36$ ) during the pretest, training and post-test phases. The error bars represent the standard error of the mean.

would be a detection problem. According to this reasoning, it is important to distinguish between asymptotic habituation and non-detectable habituation, since it has been proven that habituation often continues beyond the detection margin, which has been called "below-zero habituation" (27). Thus, to detect the possible differences between the incremental and constant conditions when below-zero habituation is produced, further investigations should employ more sensitive measures, such as the comparison of differential levels of spontaneous recovery.

Another interesting aspect of the ISIE is the type of habituation theory required to explain it. Several researchers (e.g., 13, 26), have pointed out that this phenomenon poses serious difficulties for certain habituation theories, such as the so-called comparator theory of Sokolov (24, 25). Sokolov suggested that stimulus repetition leads to the formation of a neuronal model in the cerebral cortex and that each new presentation of the stimulus is compared to the model. The greater the difference between the stimulus and the model, the greater is the expected response to the stimulus. Therefore, as these representations develop, the stimulus becomes more similar to the model, and progressively loses its capacity to produce the response. This theory fails to explain the ISIE because it assumes that the repetition of a stimulus with incremental intensities is equivalent to the repetition of different stimuli, which would always produce a difference between the model and the actual stimulus.

Groves and Thompson (13) have pointed out that their dual theory of habituation is better prepared to explain the ISIE. According to these authors, the presentation of a stimulus produces two opposite and interacting tendencies, a specific decremental tendency (or habituation, which is subordinated to the stimulus-response system) and a global incremental tendency (or sensitization, which is subordinated to the activation state or arousal of the organism), which combine to produce the observed behavior. When a stimulus is repeated, both tendencies change their magnitude depending on various factors, such as the intensity of the stimulus and the number of repetitions. The sensitization process dominates over the habituation process with more intense stimulus but decreases with the number of repetitions, while the habituation process develops more easily with lower intensities and increases with stimulus repetition. Following this logic, Groves and Thompson explain the ISIE by suggesting that the constant group suffers a higher sensitization and lower habituation than the incremental group, because the latter group receives less intense stimuli in each block.

Wagner and Vogel (31) used the associative machinery of the SOP (29) and AESOP (30) models to describe how incremental and decremental processes may interact in habituation procedures. According to the SOP model, when a stimulus is repeatedly presented in a context, the context acts as a conditioned stimulus that develops an association with the habituating stimulus, which in turns plays the role of the unconditioned stimulus. As this association develops, the stimulus becomes progressively more expected in the context. SOP further assumes that an expected or pre-processed stimulus is not as effectively processed, as it otherwise would be, which would explain the decremental tendencies that resulted from the repetition of the stimulus. On the other hand, according to the additional principles contained in the AESOP model, certain emotional responses provoked

by the stimulus, like fear, can also be conditioned to the context, which would acquire the property of potentiating the response to the habituating stimulus. The AESOP model assumes that decremental and incremental tendencies develop simultaneously and obey to different associative parameters.

A shared aspect between the dual theory of Groves and Thompson (13) and the Wagner's approach (29-31) is the assumption that the decremental process is assumed to be specific to the stimulus-response system, whereas the incremental process is global, affecting multiple response systems simultaneously. According to this, it would be possible to evaluate whether the advantage of the incremental group over the constant group is due to differential habituation or differential sensitization, if it were possible to employ two different stimuli, perhaps one acoustic and the other tactile, that have been demonstrated to have at least partially separable startle-producing features. Then it would be possible to determine whether the exposure to one of the stimuli in the incremental versus constant conditions produced less responding in the incremental condition specific to the exposed stimulus (due to differences in habituation to the repeated stimulus) or less responding to the two stimuli (due to differences in sensitization). This sort of experiments needs preparations that show robust stimulus specific habituation, which has not been demonstrated systematically yet in the procedures in which the ISIE effect has been observed (9, 28).

Another methodological strategy to uncover the real nature of the ISIE might be to examine the neural pathways of incremental and decremental processes involved in habituation procedures. This could be based in the fact that neural circuit of the startle response is clearly drawn to the level of the sensory-motor connections with the reticular system (5). It has also been demonstrated that the habituation of this response is seriously affected by lesions in this pathway (15). On the other hand, there is substantial evidence that the acquisition of different levels of sensitization occur in different neural circuit, which has been proven by studies that demonstrated that the startle response could be enhanced by the experimental activation of the amygdala (4, 6, 10). By means of the structural or chemical deactivation of one of these circuits it could be clarified if the differences between the two experimental conditions are due to differences in the habituation or sensitization circuits.

The habituation theories and the understanding of this phenomenon will be able to move beyond the current state, to the extent that some procedures are developed to allow separating the different influences that underlie this apparently simple type of learning.

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