Eyeblink cross-habituation between tactile and acoustic systems in humans

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To study cross-habituation of the eyeblink reflex in humans, subjects were repetitively exposed to a blink-eliciting stimulus in one modality and then shifted to a second stimulus in a different modality. One group of subjects began with a tactile stimulus and switched after 40 trials to an acoustic stimulus. The other began with the acoustic and switched to the tactile. No evidence of cross-habituation was found in the shift from tactile to acoustic, suggesting that habituation takes place in the sensory limb of the reflex. Cross-habituation *was* found, however, in the shift from acoustic to tactile stimuli. This result was expected because the act of blinking to the acoustic stimulus activates trigeminal afferents from the eyelid; as a result, the tactile stimulation from the blink to the acoustic stimuli had already begun habituation in the tactile pathway before the first external tactile stimulus was received.

The process of habituation occurs in all reflex pathways. Although its mechanisms are not well understood, some data suggest that habituation occurs in the sensory limb of a reflex. For example, Castellucci, Carew, and Kandel (1978) showed that in *Aplysia*, habituation of the gill withdrawal reflex results from a reduction in transmitter release from sensory neurons. The phenomenon of dishabituation, in which a new stimulus evokes a larger response than does the habituated stimulus, provides partial support for this hypothesis. Dishabituation is a ubiquitous phenomenon in reflex habituation and was included by Thompson and Spencer (1966) among criteria for true habituation. It suggests, at least, that the motor side of the reflex has not changed; a change in either the sensory or central relays is consistent with dishabituation.

The present study examined cross-habituation to investigate the locus of habituation using the eyeblink reflex, which can be evoked both by electrical stimulation of the skin overlaying the supraorbital branch of the trigeminal nerve and by acoustic stimuli. We hypothesized that habituation takes place in the sensory limb of the reflex and therefore that subjects who had habituated to one stimulus would not be habituated to a different stimulus. Thus we did not expect to find cross-habituation for the shift from tactile to acoustic stimuli. The shift from acoustic to tactile stimuli, however, presented a more complicated problem. Evinger (1995) has pointed out that the act of blinking causes air to rush through the eye-

lashes and the lid to rub across the cornea. This stimulation by itself is enough to elicit a blink, but the blink circuits are arranged so that subsequent blinks are inhibited (Pellegrini & Evinger, 1995). Powers et al. (in press) have shown that a blink elicited by an acoustic stimulus inhibits subsequent blinks elicited by tactile stimuli with the same time course, as is seen when two tactile blinks are paired, thus demonstrating that an acoustically evoked blink acts like a blink elicited by a tactile stimulus. The most parsimonious explanation of this finding is that acoustically evoked blinks activate trigeminal afferents. Because the supraorbital nerve innervates the skin of the eyelid and the skin around the eye, the act of blinking to the acoustic stimulus activates trigeminal afferents that also are activated by electrical stimulation of the skin. Thus, in the shift from acoustic to tactile stimuli in the present experiment, we expected cross-habituation to occur because the blink to the acoustic stimulus would have activated some of the same afferents that were activated by the tactile stimulus.

METHOD

Subjects

Twenty subjects (4 male, 16 female) were recruited through campus advertisement and direct recruitment in undergraduate and graduate courses.

Materials and Design

The subjects were randomly assigned to one of two groups ($n = 10$ each). One group was presented with the tactile stimulus followed by the acoustic stimulus, and this group was designated as the tactile-acoustic group. The tactile stimulus was electrical stimulation of the supraorbital branch of the trigeminal nerve. The other group was presented with the acoustic stimulus followed by the tactile stimulus and was designated as the acoustic-tactile group. By chance, all 4 males were assigned to the acoustic-tactile group.

Stimulus A was the stimulus to be presented first for 40 trials, followed by 10 presentations of Stimulus B. In order to obtain a

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measure of the prehabitation level of response to Stimulus B, it was presented twice with a 30-sec interstimulus interval (ISI) at the beginning of the experiment. This was followed by the experiment proper, which consisted of 40 trials of Stimulus A with a 5-sec ISI, followed immediately by 10 trials of Stimulus B, also with a 5-sec ISI. The subject was not informed about when the stimuli would be changed.

Stimulus presentation and data recording were performed automatically by an electromyography (EMG) data collection computer program, designed by Craig Evinger of the State University of New York at Stony Brook. Acoustic stimuli were presented by Coulbourn Instruments equipment through Realistic binaural headphones (Nova 40). The duration and rise-fall time of the acoustic stimuli were controlled by Coulbourn equipment (100 msec white noise, nearly-instantaneous rise-fall time). Tactile stimuli were presented by a WPI Stimulus Isolator (A360), and the duration of the tactile stimuli (120 μ sec) was controlled by a WPI Pulsemaster timer (A300). The level of the tactile stimulus was set at 1.5 times the stimulus threshold at which the subject showed an EMG response to the stimulus. The experimental levels of the tactile stimulus varied widely between subjects (4.0–7.0 mA), and the acoustic levels were kept constant (95 dB SPL).

The investigator attached five Grass gold-plated 8-mm surface electrodes at different points around the right eye, using Grass electrode cream (EC-2) and paper first-aid tape. Two electrodes were used to record EMG output, one affixed to the lower lid immediately inferior to the center of the right eye, and the other immediately lateral to the right eye. Two electrodes were used to present the tactile stimulus, one just superior to the supraorbital notch, and the other approximately 1 cm above and slightly medial to the first one. The fifth electrode, attached at the center of the forehead, served as a ground.

The EMGs were amplified by a differential AC amplifier (A-M Systems Model 1700, band-pass –3 dB points at 300–5000 Hz). They were collected, displayed, and stored online by a Data Translation DT2801-A board and a personal computer. The EMG was sampled at 4000 Hz; the computer rectified the record and integrated the muscle activity, and the records could be analyzed individually or averaged.

Procedure

The subjects were run in a quiet testing room (1.5 $\times$ 2.0 m; background noise level: 50 dB SPL) that contained a comfortable chair and a small table with reading material. The testing equipment was located in an adjacent cubicle.

After the subject signed a written consent form, the investigator attached the electrodes and seated the subject in the testing room. The subject was then provided with reading material and asked to read during the experiment to prevent boredom and sleepiness. The investigator adjusted the level for the tactile stimuli. The acoustic stimulus was also tested to determine whether the volume was such that it was painful. If this was the case, the volume was reduced slightly (no more than 3 dB) until it was tolerable.

The subject was then exposed to Stimulus B in the two test trials with a 30-sec ISI to establish the level of responding prior to habituation. After another pause of about 30 sec, the 40 trials with Stimulus A were presented, followed by the 10 trials with Stimulus B, all with a 5-sec ISI and no interruption. At the end of the experiment, subjects were fully debriefed regarding the rationale of the study.

For both tactile and acoustic trials, the integrated amplitude of response was calculated using a fixed window (65 msec) beginning about 15 msec after the onset of the stimulus (determined by visual inspection). In order to reduce the between-subjects variance, each subject's data total for Stimulus A was divided by the first trial on Stimulus A, and each subject's data total for Stimulus B was divided by the average of the two control trials. Because the data were not normally distributed, they were converted to log-

arithms. To determine whether habituation had occurred, Block 1 (Trials 2–10) of Stimulus A was compared to Block 4 (Trials 32–40) of Stimulus A using an analysis of variance (ANOVA). (The first trial in Block 1 could not be used because the values were all the same.) To determine whether cross-habituation had occurred, Block 1 (Trials 2–10) of Stimulus A was compared to Block 5 (Trials 42–50) of Stimulus B for the other group. Additionally, Block 4 of Stimulus A was compared to Block 5 of Stimulus B for the other group, to see whether responding increased when the new stimulus was presented. The Hunyh-Feldt correction for sphericity (ϵ) was used.

RESULTS

If intense enough, tactile stimuli typically elicit a response with two components, termed R1 and R2. In the present study, subjects rarely showed an R1 response, probably because of a weak tactile stimulus (1.5 times threshold). The results reported here for tactile responses are for R2 only.

Figure 1 shows the data for acoustic responding. Trials 1–10 and 31–40 are from the acoustic-tactile group; Trials 41–50 are from the tactile-acoustic group. As can be seen, no cross-habituation occurred: Responding on Trials 41–50 was equivalent to responding on Trials 1–10. For the within-subjects comparison of the first block of acoustic trials with the last block of acoustic trials before the shift to tactile (Acoustic Trials 2–10 and 32–40), there was a significant effect of block [$F(1,9) = 12.38, p < .01$], a significant effect of trials [$F(8,72) = 3.47, p < .01, \epsilon = .40$], and a significant interaction of blocks $\times$ trials [$F(8,72) = 3.83, p < .01, \epsilon = .66$]. Analysis of simple effects in this interaction showed a significant trials effect for Acoustic Trials 2–10 [$F(8,72) = 4.41, p < .01, \epsilon = .38$], but no trials effect for Acoustic Trials 32–40 [$F(8,72) < 1$]. These results show that habituation took place during the first block of acoustic trials. A comparison of the first block of acoustic trials with the first block of acoustic trials after the shift (Acoustic Trials 2–10 with Acoustic

Figure 1. The relative response amplitude to the acoustic stimulus across trials. For Trials 1–10 and 31–40, the amplitude of each response is divided by the amplitude of the first response. For Trials 41–50, the amplitude of each response is divided by the mean amplitude of the first two (control) responses. See text for further explanation.

Trials 42–50) showed a significant trials effect [$F(8,144) = 6.74, p < .01, \epsilon = .70$], but no difference between the groups ($F < 1$) and no significant interaction of groups $\times$ trials ($F < 1$). Thus no cross-habituation occurred. A comparison of the last block of acoustic trials before the shift with the first block of acoustic trials after the shift (Acoustic Trials 32–40 vs. 42–50) indicated a significant trials effect [$F(8,144) = 2.59, p < .05, \epsilon = .68$] and a significant interaction between groups and trials [$F(8,144) = 2.89, p < .01, \epsilon = .68$] but no significant groups effect [$F(1,18) = 1.91, p > .05$]. Analysis of the simple effects in the interaction showed that the groups differed significantly on Trials 32 and 42 [$F(1,18) = 16.73, p < .01$] and on Trials 33 and 43 [$F(1,18) = 4.87, p < .05$]. These results show that the subjects in the tactile–acoustic group performed differently when presented with the acoustic stimulus than did the subjects in the acoustic–tactile group at the end of the 40 trials of habituation.

Figure 2 presents the data for tactile responding, showing the results of Trials 1–10 and 31–40 for the tactile–acoustic group and the results of Trials 41–50 for the acoustic–tactile group. Here there was evidence of cross-habituation, as predicted. The response on Trials 1–10 was much higher than the response on Trials 41–50. Much of this difference actually seemed to result from sensitization in the first 10 trials, which was not seen following acoustic habituation. These findings were also confirmed by ANOVAs on the log data. A within-subjects comparison of tactile Trials 2–10 and 32–40 yielded a significant blocks effect [$F(1,9) = 22.69, p < .01$], showing that response amplitude was reduced by the end of the first 40 trials. There was also a significant trials effect [$F(8,72) = 3.23, p < .01, \epsilon = .68$], but no significant interaction [$F(8,72) = 1.28, p > .05$]. Comparison of tactile Trials 2–10 and 42–50 indicated a significant group effect [$F(1,18) = 6.73, p < .02$] and a significant trials effect [$F(8,144) = 3.87, p < .01, \epsilon = .92$], but no interaction between the two ($F < 1$). Thus, subjects given 40 trials of

Figure 2. The relative response amplitude to the tactile stimulus across trials. For Trials 1–10 and 31–40, the amplitude of each response is divided by the amplitude of the first response. For Trials 41–50, the amplitude of each response is divided by the mean amplitude of the first two (control) responses. See text for further explanation.

the acoustic stimulus showed smaller blinks to the tactile stimulus than did naive subjects. Finally, comparison of tactile Trials 32–40 and 42–50 yielded a significant effect for trials [$F(8,144) = 2.24, p < .05, \epsilon = .82$] but no significant effect for groups ($F < 1$) or for the groups $\times$ trials interaction [$F(8,144) = 1.37, p > .05$], showing that the subjects given tactile stimuli after acoustic stimuli performed like those given 30 previous trials of tactile stimuli.

Although the first trials of each block could not be included in these analyses, we analyzed them separately to provide an indication of whether the first trial values differed depending on whether they were preceded by habituation in the other modality. These values are presented in Table 1. If no cross-habituation occurred, Trial 1 of Stimulus A should not be significantly different from Trial 41 of Stimulus B for the other group, and Trial 40 should be significantly different from Trial 41. The results for acoustic responding conformed to this pattern. For the comparison of Trial 1 of acoustic–tactile and Trial 41 of tactile–acoustic, as expected, the difference was not significant [$t(18) = .08, p > .05$]. In addition, the difference between Trial 40 of acoustic–tactile and Trial 41 of tactile–acoustic was significant [$t(18) = -2.12, p < .05$]. The results for tactile responding, in which we observed cross-habituation in the overall analysis, were inconsistent. For the comparison of Trial 1 of tactile–acoustic and Trial 41 of acoustic–tactile, the difference was expected to be significant, because the overall analysis showed that cross-habituation occurred. This expectation was not confirmed [$t(18) = .36, p > .05$]. The difference between Trial 40 of tactile–acoustic and Trial 41 of acoustic–tactile was not expected to be significant, and it was not [$t(18) = -1.60, p > .05$].

Because of the large amount of sensitization in the tactile–acoustic group, the question arose as to whether those subjects actually showed habituation or only recovered from the early sensitization. In order to examine this issue, Dunnett's t test was used, comparing the amplitude of response on each of the last five trials with the tactile stimulus (Trials 36–40) with the amplitude of response on the first trial. In this test, both Trials 39 and 40 were significantly lower than was Trial 1 [for Trial 39, $t(45) = 2.71, p < .05$; for Trial 40, $t(45) = 2.76, p < .05$]. Thus true habituation did take place in this group.

DISCUSSION

The results showed that cross-habituation did not occur when subjects were shifted from tactile to acoustic stimuli. This finding supports the notion that habituation takes place in the sensory limb of the reflex. Even when the reflex was repeatedly elicited by the tactile stimulus, these stimuli had no effect on the acoustic pathway; thus when the reflex was elicited subsequently by acoustic stimuli, the response was not habituated.

In the overall analysis, we did find cross-habituation in the shift from the acoustic to the tactile modality, as was predicted. Analysis of the first tactile trial versus the

first tactile trial after acoustic habituation failed to show significance, however. These results suggest that the difference between the two conditions was in sensitization (after the first trial) rather than in habituation. The tactile-acoustic group showed sensitization in its responding to the tactile stimulus, whereas the acoustic-tactile group did not. If the finding of no difference between Trials 1 and 41 is correct, then a difference in sensitization may account for the whole effect. When a 5-sec ISI is used with supraorbital stimuli, sensitization typically occurs in our laboratory (unpublished observations). Similarly, in a study of habituation to supraorbital stimulation, Sanes and Ison (1983) presented evidence of sensitization of R2 when the ISI was 5 sec (their Figure 1), although they did not comment on it. Thus sensitization appears to be a reliable finding when electrical stimulation of the supraorbital nerve is given at a 5-sec ISI. In the acoustic-tactile group, however, no such sensitization occurred in responding to the tactile stimuli. An explanation for this finding is that sensitization is seen early in a series of stimuli. For the acoustic-tactile group, the acoustic trials had already provided stimulation of the trigeminal afferents from the skin around the eye, although no sensitization occurred during these acoustic trials, perhaps because the trigeminal stimulation during these trials was too weak. Thus the period when sensitization would be expected to occur was over before the first overt tactile stimulus was presented.

As noted in the introduction, the cross-habituation that occurred in the shift from acoustic to tactile stimuli was expected on the basis that eyelid closure caused by the acoustic stimulus activates trigeminal afferents from the eyelid and skin around the eye, which travel in the supraorbital nerve. Thus the tactile sensory pathway receives activation throughout the acoustic trials and showed habituation and reduced sensitization when activated directly after the shift.

Powers et al. (in press) reported results consistent with those of the present study, using paired stimuli and examining the amount of inhibition shown to the second stimulus when the first was the same or different from the second. If the two stimuli were identical (tactile-tactile or acoustic-acoustic), inhibition to the second stimulus was profound and occurred even with long ISIs (up to at least 1,500 msec). If the two stimuli were in different modalities, however, the order of presentation was critical. In the case of tactile-acoustic pairs, the inhibition did not occur if the ISI was longer than about 400 msec. This finding suggested that the long-lasting inhibition generated by two identical stimuli originated in the sensory nucleus itself: Pairs of stimuli in two different modalities generated only short-term inhibition. When the pairs were presented in the acoustic-tactile order, however, the inhibition was long lasting. Powers et al. interpreted this finding in the same way as the acoustic-tactile habituation is interpreted here: The acoustic stimulus caused activation of the trigeminal pathway when the subject blinked to it, and the acoustic-tactile condition was therefore similar to the tactile-tactile condition.

Rimpel, Geyer, and Hopf (1982) examined cross-habituation among acoustic, visual, and tactile eyeblink reflexes. They found cross-habituation when they shifted their subjects from acoustic to tactile stimuli, as was found in the present study, but they did not examine the reverse sequence, tactile to acoustic, where we found no cross-habituation. Rimpel et al., however, also found cross-habituation between modalities where we would predict that there should be none, such as acoustic to visual and visual to acoustic. In these cases, according to our hypothesis, cross-habituation should not occur because the visual pathway is not activated by the acoustic stimulus, nor is the acoustic pathway activated by the visual stimulus. Their methods were different from those of the present study: They used a habituation criterion and ran the subjects on one modality to twice the habituation criterion before shifting, and they used a 1.5-sec ISI. The most serious difference, however, and one that might be likely to cause their results to be different from ours, is that they ran the same subjects repeatedly on various combinations of stimuli, repeating each procedure five times. They did not specify the order of presentations or whether all conditions were run in a single session, but they did say that the subjects rested for "at least 10 min" between habituation sessions. It would be worthwhile to replicate their studies of cross-habituation between acoustic and visual modalities to determine whether subjects would fail to show cross-habituation if run on one procedure only.

In an earlier study on the orienting rather than startle response in rats, File and Russell (1972) suggested that cross-habituation does not occur between visual and acoustic modalities. This study used tone and light stimuli and measured disruption of licking behavior, which was disrupted at first but showed gradually less disruption as the orienting response habituated. As our results would predict, File and Russell found no cross-habituation between light and tone stimuli.

Theories of habituation fall into two main schools. One school, represented by Sokolov (1963) and Wagner (1979), among others, attributes habituation to the formation of a template of the stimulus, to which the organism matches the incoming stimulus. If the incoming stimulus matches the template, responding to it is reduced. This theory would predict that a new stimulus (say, an acoustic stimulus after tactile habituation) would not match the template and would therefore not lead to cross-habituation. The results of the tactile-acoustic group in the present study are consistent with this prediction. The finding that cross-habituation did occur in the shift from acoustic to tactile stimuli would not have been predicted by these sorts of theories, but presumably could be explained as we have explained it above by the assumption that acoustic stimuli cause activation of the tactile pathway. Thus two forms of trigeminal stimulation, indirect when the subject blinks to an acoustic stimulus and direct when the skin is stimulated electrically, both activate the same template. In addition, however, acoustic stimuli activate an acoustic template. If the tactile template has already been activated by acoustic stimuli, the subject will show

Table 1
Mean Amplitude (With Standard Deviations) for Selected Trials

Group	Trial 1		Trial 40		Trial 41	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Acoustic-tactile	28,373	17,951	9,315	8,617	23,947	22,464
Tactile-acoustic	28,550	31,519	10,771	10,112	27,572	24,329

habituation to a direct tactile stimulus. The reverse is not true, however, because the acoustic stimuli activate the acoustic template for the first time when they are presented after tactile stimuli.

The other school, represented by Thompson and his associates (Groves & Thompson, 1970; Thompson & Spencer, 1966), presents the phenomenon of response habituation as a result of the combination of two processes: One first increases and then decreases with repeated presentations (sensitization), and the other decreases steadily over the stimulus train (habituation). According to Groves and Thompson, habituation takes place in the reflex pathway itself whereas sensitization takes place outside the pathway. They stated that both inferred processes, habituation and sensitization, will generalize to a test stimulus to the extent that the test stimulus activates common elements with the original stimulus. Thus cross-habituation (or cross-sensitization) will take place to the extent that the stimuli activate common elements. For the results of the tactile-acoustic group, the tactile and acoustic stimuli did not activate common elements and therefore cross-habituation did not occur. A strict reading of the theory might predict that cross-habituation should not occur when the order of presentation was reversed, since the issue is whether the same elements are activated by both stimuli. For the acoustic-tactile group, however, these theorists could presumably accommodate the results by using the explanation given above—that the acoustic stimulus activates trigeminal afferents when the subject blinks, so that when the trigeminal stimulus is eventually presented, it activates elements that have already been activated by the previous stimulus.

Neither theory is inconsistent with the results obtained here; nor do these two theories make differential predictions about cross-habituation. Nevertheless, theorists have yet to address specifically the issue of where in the reflex pathway habituation occurs. The present results lend support to the hypothesis that habituation is a sensory phenomenon.

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