

EVOKED POTENTIAL DECREMENTS IN AUDITORY CORTEX. II. CRITICAL TEST FOR HABITUATION *

I.S. WESTENBERG ‡ and N.M. WEINBERGER

Department of Psychobiology, School of Biological Sciences, University of California, Irvine, Irvine, Calif. 92717 (U.S.A.)

(Accepted for publication: October 9, 1975)

Decrements in the amplitude of evoked potentials (EPs) recorded from the auditory cortex in response to iterated acoustic stimuli can be called habitatory only after all other possible explanations of the decreases have been ruled out. For example, the hypothesis that decreases are due to some change in the stimulus at the receptor has been ruled out by demonstration of decrements in evoked potentials in paralyzed cats, where stimulus variability was controlled (Horvath 1969; Westenberg et al. 1976). Another hypothesis suggests that the decrements reflect a change in state (Teas and Kiang 1964; Thompson and Spencer 1966; Petrek et al. 1967, 1968; Sommer-Smith and Morocutti 1970). The gist of this hypothesis is that during the course of a monotonous stimulation experiment, the state of the subject changes such that EPs *in general* become reduced. The most direct means of ruling out the state hypothesis of a general change in EPs is to show an EP decrement that is selective and specific to the repetitive stimulus. In other words, it must be shown that while there is a decrement in the EPs to the repetitive stimuli, there is less (or

no) decrement in the EPs to “test” stimuli that differ from the repetitive stimuli along some dimension. The minimum requirement for such an experiment would be two stimuli “X” and “Y”, e.g., tone pips differing along the dimension of frequency (Key 1965). In this case, one of the stimuli would be high and one would be low; one of these would be the repetitive stimulus and the other would be the test stimulus. When “X” is the repetitive stimulus, a reduction in the EPs to “X” that is larger than the reduction (if any) in the EPs to test stimulus “Y” would show that the “X repetitive” EP decrement is selective.

However, such results could be attributable to either of two independent variables, i.e., the selective decrement could be specific to the frequency or the repetitive aspect of “X”. Since there are two independent variables (high vs. low and repetitive vs. test) a counter-balanced design is required. Thus, in a complementary part of the experiment stimulus “X” must be the test stimulus, and “Y” must be the repetitive stimulus. Then there must be a reduction in the EPs to “Y”, and there must *not* be a larger reduction in the EPs to “X”. Such results would exclude the possibility that whenever EP decrements occur, the reduction in the EPs to pips of “X’s” frequency is always larger than the reduction in the EPs to pips of “Y’s” frequency, regardless of which stimulus is repetitive. This would rule out the argument that the selective “X repetitive” EP decrement was a function of the particular

* This research was supported in part by Mental Health Training Grant MH 11095.

‡ Supported by Mental Health Training Grant MH 11095 and a Predoctoral Fellowship from the National Institute of Mental Health. Present address: 183-A, Veterans Administration Hospital, 7th Street and Indian School Road, Phoenix, Ariz. 85012.

frequencies of "X" and "Y". By elimination, the repetitive aspect of "X" would be left as the essential variable in the "X repetitive" EP decrement. Thus the "X repetitive" EP decrement would be shown to be both selective and specific to the repetitive stimulus.

The present experiments employed tone pips as the repetitive and test stimuli. The main experiment was supplemented by a study that provided an opportunity to assess the reliability of the findings. Also, as in previous experiments (Westenberg et al. 1976) there were controls for stimulus constancy plus detailed recording and analysis of data, with statistical tests of results. The experiments were designed to allow rejection of the hypothesis that decrements in EPs to continually repeated stimuli are part of a non-specific change in EPs. Thus, they provided an opportunity to observe decrements in cortical EPs that were conclusive demonstrations of habituation.

Methods

Subjects

Subjects were 5 adult cats (3 male, 2 female, 3.4–5.2 kg). All (F-5, -7, -8, -11 and -12) were used in the main experiment, and three (F-5, -7 and -8) were used in the supplemental experiment.

Stimulation

Stimuli were tone "pips" formed from a sine wave produced by a Wavetek Voltage-Controlled-Generator. The frequency of the sine wave could be changed precisely and instantly by switching the input voltage to this instrument. The pips were formed by a Grason-Stadler audio switch. The duration of the pips was 100 msec with rise and decay times of 2.5 msec, to avoid onset and offset "click" artifacts. The pips were fed to Hewlett-Packard 350-D attenuators and a matched pair of TDH-39 earphones. Straight hollow metal tubes led from the earphones to the ex-

ternal auditory meati of the subjects. The intensity of the pips at the ends of the tubes was 75 dB (re: 0.0002 dynes/cm², as measured by a Bruel and Kjaer Impulse Sound Level Meter). The pips were programmed to be presented at a rate of 1/sec by Tektronix waveform and pulse generators. BRS "digibit" programming modules were used to effect changes in the pip frequency.

Methods of electrode implantation and of recording were identical to those in the companion experiments (Westenberg et al. 1976).

Pre-experimental procedure

One week prior to the actual experiment, the subjects were paralyzed by injection of 10 mg/kg (i.p.) of gallamine triethiodide (Flaxedil). With topical local anesthesia (Cetacaine), a patent airway was established with a pediatric endotracheal tube (coated with Xylocaine jelly), and artificial respiration was begun. The respirator was set to maintain a stable expired CO₂ level and pupillary responsiveness to auditory, visual and tactile stimuli. A modified stereotaxic frame was used to hold the subject atraumatically in an isolation chamber (Industrial Acoustics). Supplemental injections of Flaxedil were administered as needed. The subjects were exposed to sets of 50–100 tone pips; a single pip frequency was used for each set, with frequencies varying from set to set from 200 to 9600 c/sec. The subjects were then allowed to recover from the paralysis. The purpose of this procedure was to verify that EPs to different tones could be recorded from each electrode while the subject was unanesthetized and paralyzed.

The main experiment

At the outset of the experiment the subject was again paralyzed and prepared as above. The experimental paradigm is outlined in Fig. 1. One frequency was chosen for the iterated stimulus and designated REPETI-

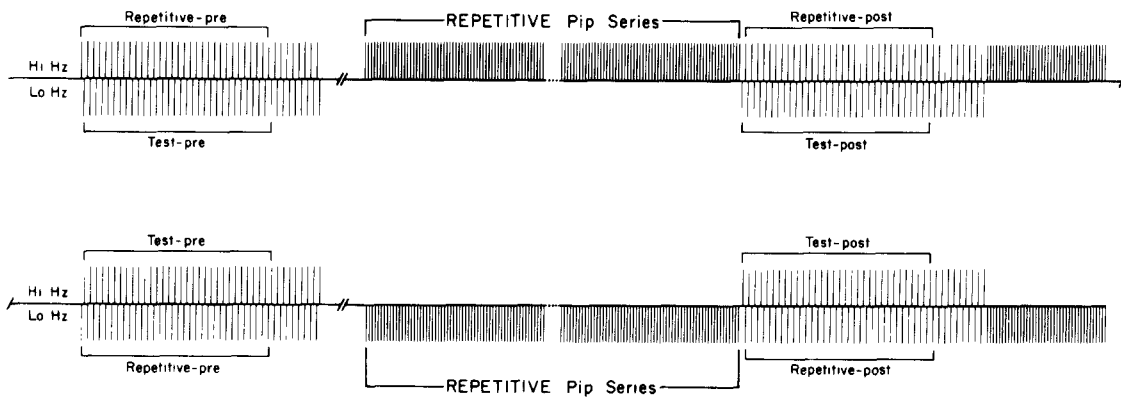


Fig. 1. Paradigm for the main experiment. Each vertical bar represents a tone pip; Hi Hz and Lo Hz refer to the high frequency and low frequency pips respectively. REPETITIVE-pre and TEST-pre indicate the pips for which evoked potentials were collected and averaged to obtain pre-iterated-stimulus-series average evoked potential (AEP) measures for the repetitive and test stimuli respectively; REPETITIVE-post and TEST-post indicate the pips for which evoked potentials were collected and averaged to obtain post-iterated-stimulus-series AEP measures for the repetitive and test stimuli respectively. REPETITIVE pip series indicates the 15 min period of repetitive pip presentation; all stimulation was at the rate of 1/sec. Note that the part of the experiment in which the high pips were repetitive and the low pips were the test stimuli complements the other part (low-repetitive, high-test). Note also that the TEST-pre stimuli were alternated (1 : 1) with the REPETITIVE-pre stimuli and the TEST-post stimuli were similarly alternated (1 : 1) with the REPETITIVE-post stimuli.

TIVE; another frequency was chosen for the test stimulus and designated TEST. After a 5 min isolation period, 32–64 REPETITIVE pips and an equal number of TEST pips were presented in an alternating fashion (i.e., 1 REPETITIVE – 1 TEST – 1 REPETITIVE – 1 TEST . . .) at a rate of 1/sec. The pips were to obtain pre-iterated-stimulus-series EPs, designated “REPETITIVE-pre” and “TEST-pre”, respectively. After 5 more minutes of quiet, a series of 900 REPETITIVE pips were presented at 1/sec. Then, with no interruption in the regular presentation rate, 32–64 REPETITIVE pips and an equal number of TEST pips were again presented in an alternating fashion. These were to obtain post-iterated-stimulus-series EPs designated “REPETITIVE-post” and “TEST-post”, respectively. It should be noted that the REPETITIVE-pre EPs were collected during the same time period as the TEST-pre EPs, just as the REPETITIVE-post EPs were collected during the same time period as TEST-post EPs. Thus, unless the subject’s state was changing at a rate of once per sec, it can be

assumed that the state during the recording of the REPETITIVE-pre EPs was essentially the same as the state during the recording of the TEST-pre EPs. Likewise, it can be assumed that the state during the recording of the REPETITIVE-post EPs was essentially the same as the state during the recording of the TEST-post EPs.

After a check on the subject during a 15 min break period with no tone stimulation, the entire procedure was repeated with the frequencies designated REPETITIVE and TEST reversed. In other words, the frequency that initially had been chosen for the iterated stimulus was made the test stimulus and the frequency that initially had been chosen for the test stimulus was made the iterated stimulus. Subjects were then allowed to recover from paralysis. Later, electrode placements were verified as in the companion studies (Westenberg et al. 1976).

Data analysis

The data for each brain recording site were

computer averages of EPs. Usually a group of 32 EPs was divided into 8 subgroups of 4 EPs; the *group* averages were used for all analyses except where indicated below. The average evoked potential (AEP) consisted of 106 points; each point represented the average voltage at some point in time (0–105 msec) after the pip onset. Each AEP was plotted by the computer. One AEP was obtained for each set of REPETITIVE-pre, REPETITIVE-post, TEST-pre and TEST-post EPs. During each series of 900 pips, one sample of 32 consecutive EPs was averaged approximately every 45 sec; generally, 20 samples were taken over the 15 min (900 pip) period. As in the companion experiments (Westenberg et al. 1976), systematic AEP changes during the 900 pip series were assessed by amplitude-(or latency)-by-sample-number correlation coefficients. Changes during three time periods, minutes 1–5, 1–10 and 1–15, of the iterated stimulus series were tested. The three time periods were chosen to detect significant changes with faster (within the first 5 min) as well as slower (within 10 or 15 min) time courses. The criterion of a systematic change was a correlation coefficient with $P < 0.05$ for at least one of these time periods. Other statistical tests were applied, as indicated in the Results.

Criteria for habituation

In order to lend some direction to the consideration of the analyses and results, let us consider the goal at hand. We wish to rule out the possibility that the reduction in the EPs to a particular repetitive tone-pip stimulus, "X", is part of a general non-selective reduction in EPs due to a state change. In order to do so, the following results must be obtained.

1. When the stimulus in question, "X", is repeated, its AEPs should become reduced during the iterated stimulus series. Also, there must be a pre-post decrease in the "X" AEPs and a smaller pre-post decrease (if any) in the AEPs to a test stimulus, "Y", a tone pip

of another frequency *. To rule out the possibility that whenever AEPs decrease, the AEPs to pips of "X's" frequency always decrease more than the AEPs to pips of "Y's" frequency, the following additional results must be obtained in the complementary part of the experiment.

2. When "Y" is repeated, and the stimulus in question, "X", is the test stimulus, AEPs to "Y" during the repeated stimulus series must decrease, and the pre-post decrease (if any) in the "X" AEPs *must not be greater* than the pre-post decrease in the "Y" AEPs. By exclusion of alternative possibilities, the repetitive aspect of "X" would remain as the critical variable in the decrement in (1) above; the decreases in the AEPs to repetitive stimulus "X" could *not* be attributed to a state-related general change in EPs.

Given these criteria, we can derive a set of conditions that must be met by the data. (a) The first condition is a decrease in the AEPs to the repetitive stimulus during *both* repetitive stimulus series. A decrease during the repetitive stimulus series in one part of the experiment is required by (1) above. A decrease during the repetitive stimulus series of the complementary part of the experiment is called for in (2) above. (b) The second condition is a REPETITIVE pre-post decrease and a smaller TEST pre-post decrease (if any) in one part of the experiment. This is required by (1) above. In the complementary part of the experiment the TEST pre-post decrease (if any) should *not* be larger than the REPETITIVE pre-post decrease; this is required by (2) above. Thus, in neither part of the experiment should the TEST pre-post decrease be larger than the REPETITIVE pre-post decrease.

Any component of the AEPs to repetitive stimuli satisfying conditions (a) and (b) may be said to have demonstrated a *selective* and

* The "pre-post decrease" refers to a comparison of the "REPETITIVE-pre" vs. "REPETITIVE-post" AEPs or the "TEST-pre" vs. "TEST-post" AEPs.

specific amplitude reduction. This is an amplitude reduction that may justifiably be called habituation and not a general state-related change in EPs.

Results

EPs were successfully recorded from all 4 electrodes in each of the 5 subjects; thus there were 20 recording loci (Fig. 2).

Amplitude decrements

a. During the repetitive stimulus series systematic decrements in AEP amplitude were frequently observed*. Of 124 peak-to-peak components analyzed, 18 (15%) showed amplitude decreases in *both* of the iterated stimulus series. These were observed at 12 loci in 4 subjects.

b. REPETITIVE pre-post decreases and corresponding TEST pre-post differences. The REPETITIVE pre-post change in the

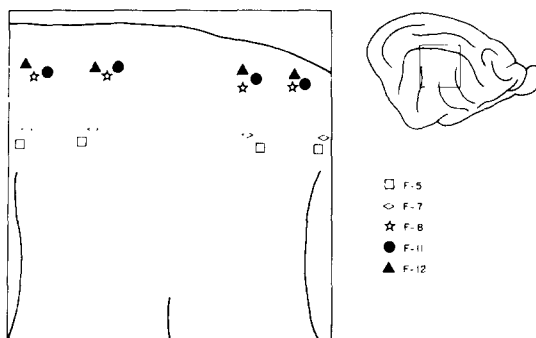


Fig. 2. Cortical map indicating electrode placements for all subjects.

* Four components of subject F-12 exhibited large decreases in amplitude that were very rapid during both repetitive pip series ($\geq 75\%$ of the corresponding mean amplitude from sample 1 to sample 2, and near asymptote thereafter). Although these decrements were too rapid to be detected by the correlation criterion, they were included as systematic decrements.

amplitude of each component meeting condition (a) was quantified by measuring (by computer) the amplitude of the appropriate component in the REPETITIVE-pre AEP and in the REPETITIVE-post AEP. The difference between the two was expressed as a percentage of the REPETITIVE-pre amplitude. Similarly, the TEST pre-post change in the amplitude of the component in question was quantified by measuring (by computer) the amplitude of the appropriate component in the TEST-pre AEP and in the TEST-post AEP. The difference between the two was expressed as a percentage of the TEST-pre amplitude.

The REPETITIVE pre-post changes usually were decreases; there were fewer TEST pre-post decreases, and these were usually smaller. This was evident when the "pre" AEPs were superimposed on their corresponding "post" AEPs. For example, in the case of subject F-5, when the high frequency tone pip was repetitive, there were REPETITIVE pre-post decreases in components "f-g" and "g-h" (Fig. 3, A) and smaller TEST pre-post decreases in "f-g" and "g-h" (Fig. 3, B). These results suggested that the response decrement was selective. In the complementary part of the experiment, when the low frequency tone pip was repetitive, there again were REPETITIVE pre-post decreases in "f-g" and "g-h" (Fig. 3, C) and smaller TEST pre-post decreases in "f-g" and "g-h" (Fig. 3, D). Such results ruled out the frequencies of the pips as the critical variable in the decrements. Note that there were larger pre-post decrements in the last two components of the AEPs to the high tone pip when it was repetitive (Fig. 3, A) than when it was the test stimulus (Fig. 3, D). Similarly, the pre-post decrements in the last two components of the AEPs to the low tone pip were larger when it was repetitive (Fig. 3, C) than when it was the test stimulus (Fig. 3, B). Frequency could not have been the critical variable here, because the pre-post decreases in "f-g" and "g-h" were larger in the REPETITIVE AEPs than in the TEST AEPs, regardless of which

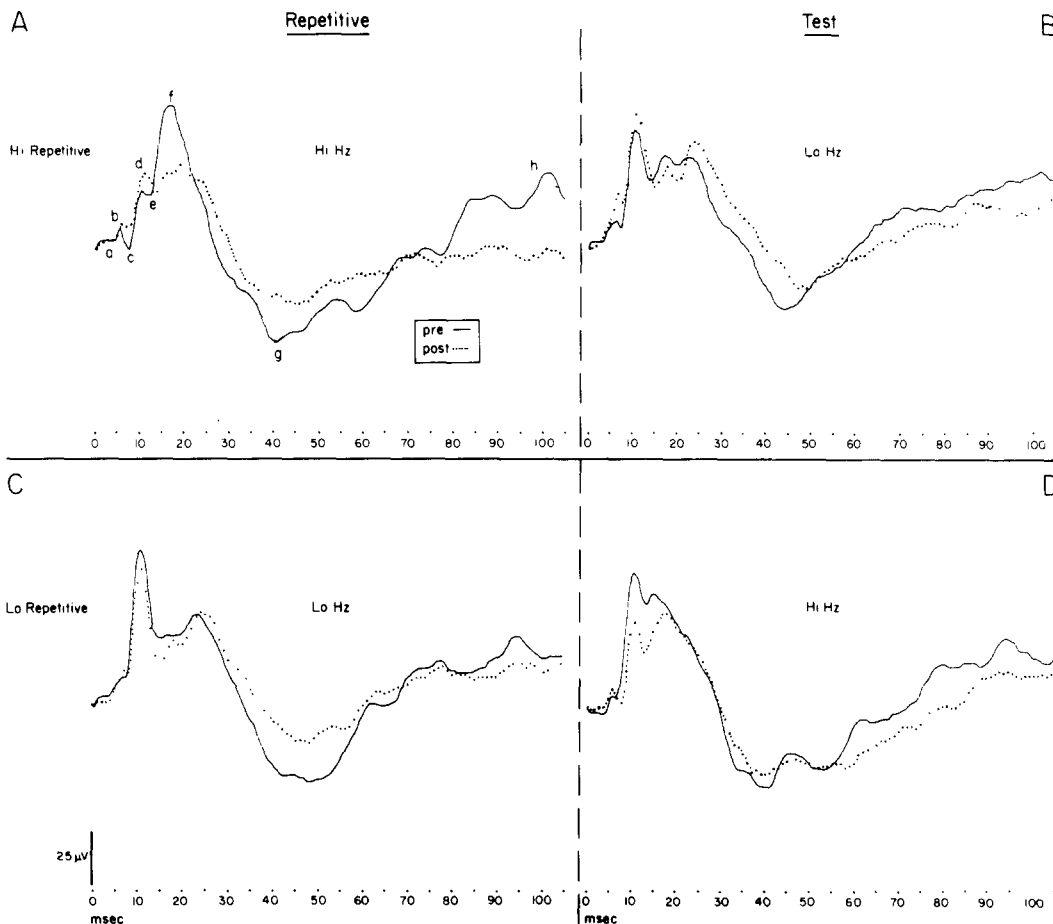


Fig. 3. AEPs for subject F-5, anterior locus. *A* and *C*: REPETITIVE-pre AEPs (solid line) contrasted with the REPETITIVE-post AEPs (dotted line). *B* and *D*: TEST-pre AEPs (solid line) contrasted with the TEST-post AEPs (dotted line). Results are for both parts of the main experiment ("Hi Repetitive" and "Lo Repetitive"); the AEP constituents that were analyzed are labeled "a" through "h"; Hi Hz = 3.2 kc/sec; Lo Hz = 1.0 kc/sec. Components f-g and g-h met all of the criteria for habituation. AEP is average of 28 evoked potentials. Positive is up in this and Fig. 4 and 6.

frequency was repetitive. The decrements were not general but rather were selective and specific to the repetitive stimulus.

Essentially the same results were obtained in the case of subject F-12 (posterior recording site), one of the subjects that participated in the main experiment, but not in the supplemental experiment. In this case, the component that decremented during both repetitive-pip series was the last component, "i-j" (latency 60-105 msec). When the high frequency tone pip was repetitive, there was a

large REPETITIVE pre-post decrement in "i-j" (Fig. 4, *C*) while there was actually a large TEST pre-post increase in "i-j" (Fig. 4, *D*). In this case the decrement certainly was not general. In the complementary part of the experiment, when the low frequency pip was repetitive, there again was a large REPETITIVE pre-post decrease in "i-j" (Fig. 4, *A*) while there was almost no TEST pre-post decrease at all in "i-j" (Fig. 4, *B*). As in the case of subject F-5, the frequencies were not critical. There was a large pre-post decrease in

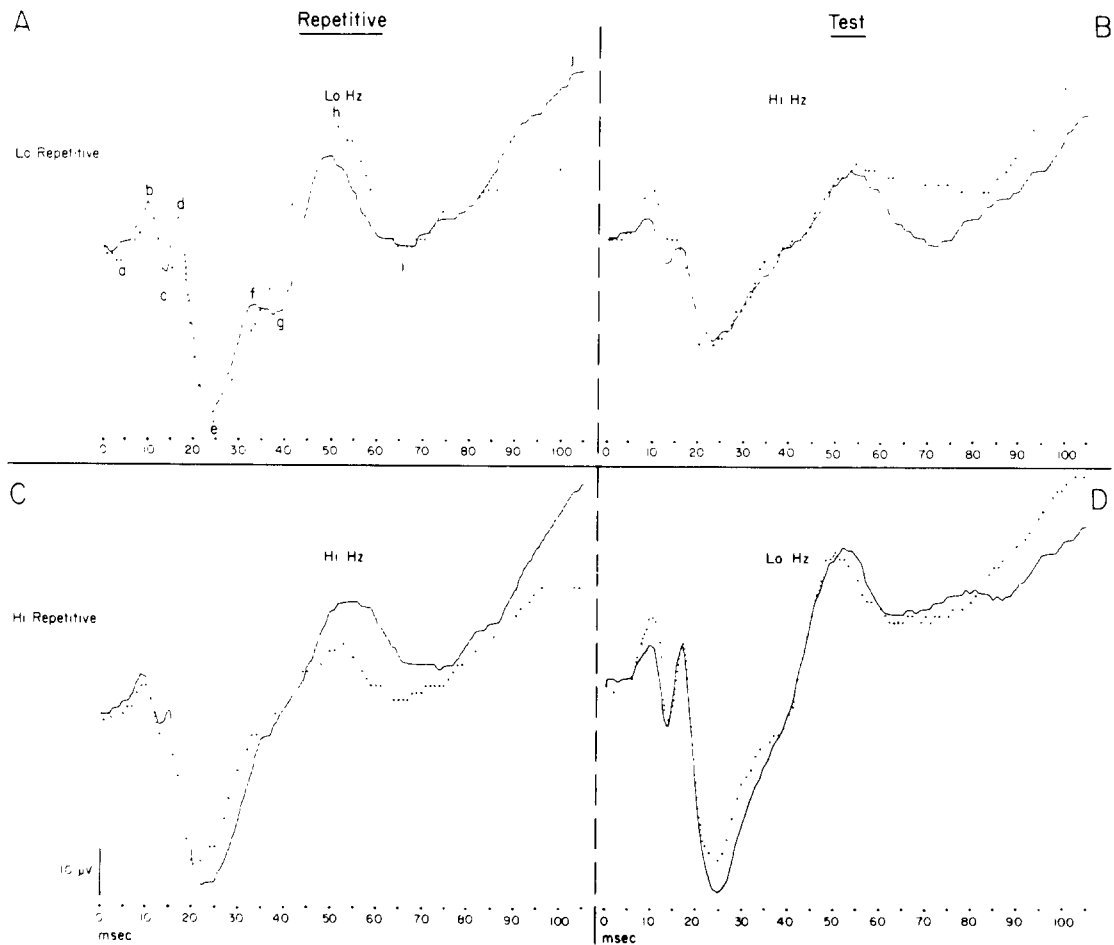


Fig. 4. Pre-post comparisons for subject F-12, posterior recording site, main experiment; Lo Hz = 2.8 kc/sec; Hi Hz = 6.4 kc/sec. Note that for this subject the low frequency pip was the repetitive stimulus in the first part of the experiment (A); otherwise comparable to Fig. 3. Component i-j met all the criteria of habituation. AEP is average of 40 evoked potentials. Solid line AEP = "pre"; dotted line AEP = "post".

"i-j" in the case of the REPETITIVE AEPs and virtually none in the case of the TEST AEPs, regardless of which frequency was repetitive; the selective decrements again were specific to the repetitive stimulus.

Of the 18 components for which decrements were found during both repetitive stimulus series, 13 (72%) showed REPETITIVE pre-post decreases and smaller corresponding TEST pre-post decreases (if any) in both parts of the experiment. To determine if these REPETITIVE pre-post decreases were statistically significant, the following additional

analysis was performed. The corresponding component of the REPETITIVE-pre subgroup AEPs was compared to the same component in the REPETITIVE-post subgroup AEPs by the Mann-Whitney U test (Siegel 1956); this was done for both repetitive-pip series. Eleven of the 13 components (85%) that were analyzed to this point showed significant REPETITIVE pre-post decrements in both parts of the main experiment. To determine if the TEST pre-post decrements (when they occurred) were also significant each component for which both REPETITIVE pre-post

TABLE I
Habitatory amplitude decrements.

| Subject locus * | msec | | | | | | | | | | | |
|-----------------|------|---|----|----|----|----|-------|-------|-------|-------|-------|-------|
| | | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
| F-5 | (1) | | | | | | ----- | ----- | ----- | ----- | ----- | ----- |
| | (2) | | | | | | | | ----- | ----- | ----- | ----- |
| | (3) | | | | | | | | | ----- | ----- | ----- |
| | (4) | | | | | | | | | | ----- | ----- |
| F-7 | (1) | | | | | | | | | | | |
| | (2) | | | | | | | | | | | |
| | (3) | | | | | | | | | | | |
| | (4) | | | | | | | | | | | |
| F-8 | (1) | | | | | | ----- | ----- | ----- | ----- | ----- | ----- |
| | (2) | | | | | | | | | | | |
| | (3) | | | | | | | | | | | |
| | (4) | | | | | | | | | | | |
| F-11 | (1) | | | | | | | | | | | |
| | (2) | | | | | | | | | | | |
| | (3) | | | | | | | | | | | |
| | (4) | | | | | | ----- | ----- | ----- | ----- | ----- | ----- |
| F-12 | (1) | | | | | | | | | ----- | ----- | ----- |
| | (2) | | | | | | | | | | ----- | ----- |
| | (3) | | | | | | | | | | | ----- |
| | (4) | | | | | | | | | | | ----- |

* |-----| = Component meeting all criteria of habituation.
 | = Component failing to meet all criteria of habituation.

comparisons were significant was analyzed further. The corresponding component in the TEST-pre subgroup AEPs was compared to the same component in the TEST-post subgroup AEPs by the Mann-Whitney U test (Siegel 1956); this was also done for both repetitive-pip series. All 11 of the components so analyzed failed to show a significant TEST pre-post change in at least one part of the experiment. Therefore, it was concluded that 11

components met all of the criteria of habituation.

All of these results were observed in peak-to-peak components whose constituent peaks had latencies ≥ 17 msec; there were 68 such longer-latency components altogether. Thus, 16% of later components habituated, while none of the initial components did. These results are summarized in Table I.

Other findings

Amplitude increments

Systematic increments in AEP amplitude were also observed, although less frequently than were decrements. Of the 124 components analyzed, 8 (6%) showed amplitude increases in both iterated stimulus series. However, none of these components met a criterion analogous to condition (b) above.

Latency changes

Systematic increases in peak latencies during both parts of the experiment were observed in 9 of 144 (6%) of the peaks. Three of the 11 components that habituated had at least one constituent peak showing latency increases. However, none of these peaks met a criterion analogous to condition (b) above. Systematic decreases in peak latency during both parts of the experiment were also observed in 9 of the 144 peaks analyzed (6%). Again, 3 of the 11 components showing an habituatory amplitude decrement had at least one constituent peak showing latency decreases, and again none of these met a criterion analogous to condition (b) for habituatory amplitude decrements.

Reliability of decrements

It is noteworthy that the design of the main experiment could have led to dishabituation, because the "post" series (immediately after the repetitive-pip series) included novel tone pips (the test stimuli) alternating with the repetitive pips (Fig. 1). Such dishabituation would have masked habituatory decrements. However, as seen above, many habituatory decrements were found. Given the risk of dishabituation, a precautionary supplemental experiment preceded the main experiment for subjects F-5, -7 and -8. The design was similar to the main experiment except that tone pips of the "pre" and "post" series did not alternate but were presented in blocks (Fig. 5). Thus there was no risk of dishabituation because all of the REPETITIVE-post EPs were collected prior to the presentation of the novel TEST-post stimuli. Although the masking effect of dishabituation did not materialize, the results of this supplemental experiment are of interest because they provide an opportunity to determine the reliability of the habituatory decrements found in the main experiment.

For the 3 subjects run in both the main and

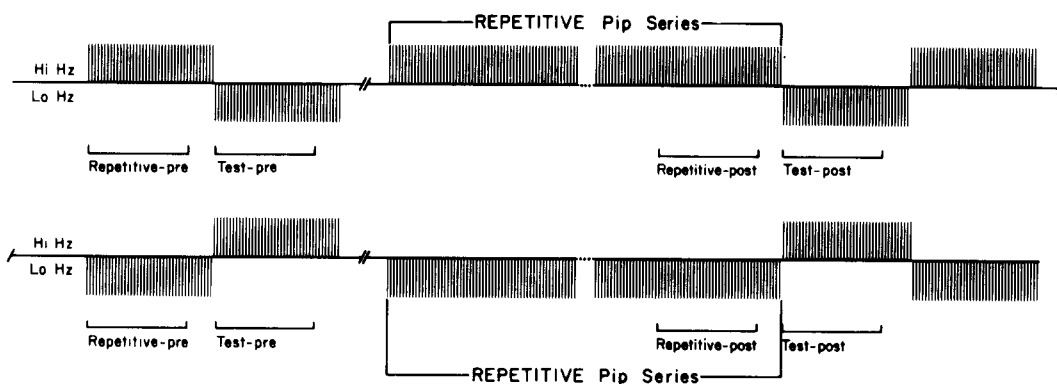


Fig. 5. Paradigm for the supplemental experiment; all terms as in Fig. 1. Note that the TEST-pre stimuli were presented in a block immediately after the block of REPETITIVE-pre stimuli. The last EP sample of the 900 pip REPETITIVE pip series provided the REPETITIVE-post AEP; the TEST-post stimuli were presented in a block immediately after the 900 pip series. Otherwise, the paradigm is the same as that of the main experiment.

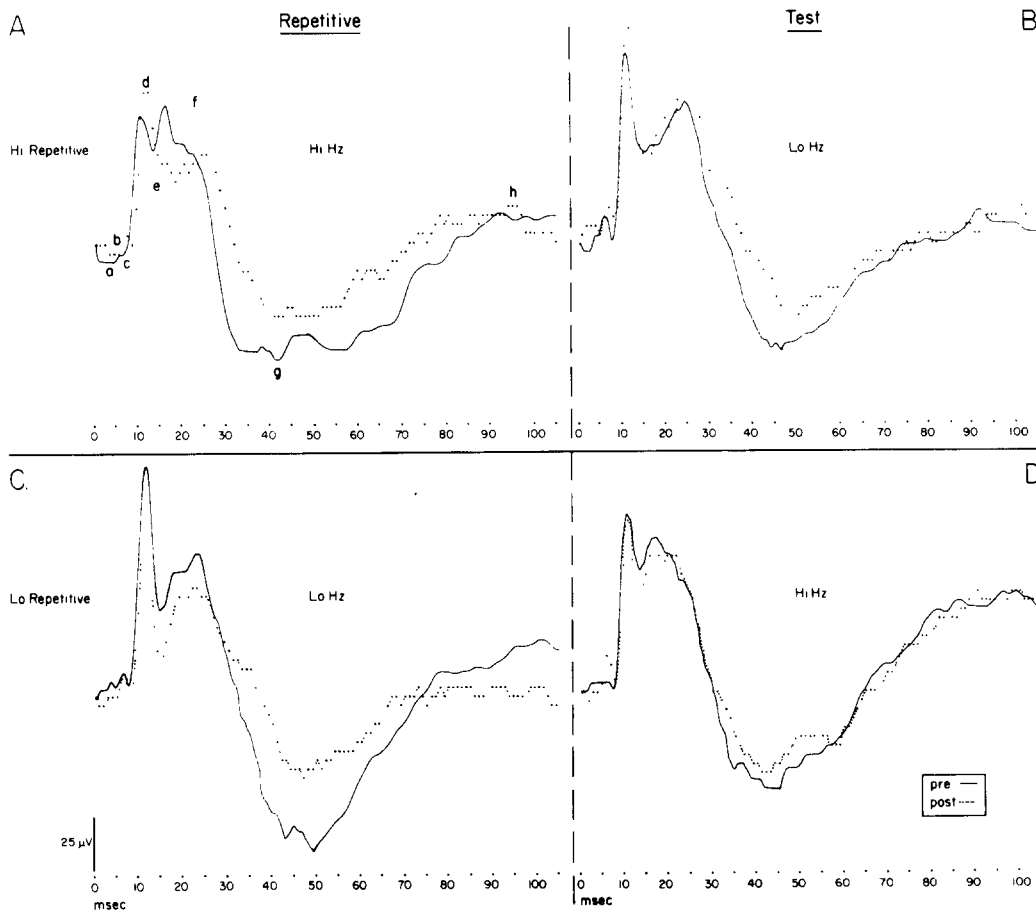


Fig. 6. Pre-post comparisons for the supplemental experiment for subject F-5. As in the main experiment, components f-g and g-h met all the criteria of habituation; compare with Fig. 3. AEP is average of 32 evoked potentials. Solid line AEP = "pre"; dotted line AEP = "post".

supplemental experiments, 68 evoked potential components were analyzed. Six components exhibited habituary decrements in the main experiment and 6 components met the criteria of habituation in the supplemental experiment; 4 of these showed habituary decrements in both experiments. Given the number of habituary decrements in the main and supplemental experiments, the probability of obtaining this much overlap in the results by chance is less than 0.001 (by combinatorial enumeration, Feller (1968)). Examples of habituary decrements in the supplemental experiment are provided for subject F-5 in Fig. 6. Components "f-g" and "g-h"

exhibited selective decrements; the same components had habituary decrements in the main experiment for subject F-5 (Fig. 3). The results of the supplemental experiment indicate the reliability of habituary decrements in certain components of AEPs.

Discussion

The question addressed at the outset of the present studies was whether decrements in the amplitude of cortical evoked potentials during repetitive acoustic stimulation were in fact habituary or not. We defined an habit-

uatory decrement as one which was both selective and specific to the repetitive stimulus. This definition was used to derive the particular phenomena that would have to be observed in order to accept decrements as habituary rather than reflective of non-specific processes such as state changes. The results of the main experiment demonstrated that some of the decrements in later components of cortical AEPs (latency 17–105 msec) were in fact habituary. The results of the supplemental experiment bolstered this conclusion.

The first question that must be asked of any set of positive findings is the probability of obtaining results as good as or better than the observed results simply by chance. If the systematic changes that we are calling examples of habituation were simply chance occurrences then one might expect there to have been as many amplitude increases as decreases meeting our rather strict criteria. In the main experiment there were 11 components demonstrating amplitude changes which met our criteria; all of these were amplitude decreases. Given that there were 11 cases of amplitude changes, the probability that, by chance, *all* would be in the same direction is less than 0.001 (sign test, Siegel (1956)).

However, alternative explanations must be considered. For example, it might be argued that the absence of significant pre–post decrements in the TEST AEPs reflects a “floor effect”, that is, the TEST-pre AEPs were so small that they could not become significantly smaller. This possibility may be discounted, because there were pre–post decreases to the same stimuli when they were used as repetitive stimuli.

Another explanation of the present results might be applied in cases of REPETITIVE and TEST pre–post decrements where the percentage change was larger for the REPETITIVE AEPs than for the TEST AEPs. If the REPETITIVE-pre value was less than the TEST-pre value, it could be argued that the largeness of the percentage of the REPETITIVE pre–post change was misleading, i.e., that the absolute change was small but ap-

peared large relative to the small REPETITIVE-pre value (Hinde 1970). This argument would be ruled out in those cases where the absolute change as well as the relative change was larger for the REPETITIVE rather than the TEST AEP. In fact, one case in the main experiment was potentially vulnerable to the above argument. However, in that case the absolute change was larger for the REPETITIVE than the TEST AEP; thus the argument does not apply to the present results.

There is one other explanation of the present findings which we cannot rule out at this time. An assumption made at the outset of the main experiment was that state would not change continually at a rate of once per sec. However, the alternative hypothesis that such changes were taking place cannot be rejected on the basis of our data. It is conceivable that with regularly alternating stimuli there are short time course “anticipatory” non-specific changes of state. Future experiments with randomized presentation of stimuli should settle this issue.

Definition of habituation

Our conclusion that decreases in some EP components were habituary was based on our definition of habituation as selective response decrement specific to the repetitive stimulus. This definition of habituation is more complex than that applied in most previous studies of EPs in the auditory cortex, where any decrease was called habituation. In the past, little or no consideration has been given to controls that might establish the decrements as selective changes in response indicative of the animal's experience with the particular repetitive stimulus. A noteworthy exception in Key's (1965) report of decreases in EPs to repetitive tone pips that exceeded changes in the EP to a “test” tone pip of another frequency. His observations were apparently too few to test statistically and he failed to control for stimulus constancy. Furthermore, he did not use a counterbalanced design to discriminate between

the two independent variables: the high vs. low (frequency) aspect and the repetitive vs. test aspect of the pips. Thus Key provided the only prior evidence of selective decrements in cortical EPs to acoustic stimuli; however, he did not establish that the decrements were specific to the repetitive stimulus.

The importance of investigating other characteristics of response decrements has been emphasized by Thompson and Spencer (1966), who derived an empirical definition of habituation in terms of nine of the most commonly observed characteristics of behavioral response decrements. Although our definition of habituation was derived differently, it has elements in common with that of Thompson and Spencer. The first, of course, is response decrement with repetitive stimulation. This is common to all definitions of habituation. The second is stimulus generalization; the present experiments are abbreviated stimulus generalization experiments.

However, in our experiments *failure* to observe evidence of generalization, i.e., a decrease in the response to the repetitive stimulus with no change in the response to the test stimulus, is the strongest evidence that the decrease is specific to the repetitive stimulus rather than part of a non-specific change in EPs. Furthermore, *complete* generalization in both parts of the present experiment, i.e., a decrease in the response to the repetitive stimulus and an equal decrease in the response to the test stimulus, would have been inconclusive. It would not allow rejection of either the habituation or the state hypothesis. Thus, although our experiment was analogous to a stimulus generalization experiment, we only considered a small amount of generalization or *no* generalization as a "positive" result.

The other elements of Thompson and Spencer's definition of habituation are less important because they do not rule out the state hypothesis. Dishabituation in the absence of other control experiments is not a critical test of either the habituation or state hypothesis (Thompson and Spencer 1966; Groves and Thompson 1970); thus, neither is

"habituation of dishabituation". "Spontaneous recovery", savings ("potentiation of habituation"), rate effects, and "below zero" effects also do not discriminate between the habituation and state hypotheses. Intensity effects (more habituation with weaker stimuli), like dishabituation, are attributed to non-specific changes ("sensitization") in "state systems" by Groves and Thompson (1970). Thus the present studies did not include tests for these "parametric characteristics". The phenomena observed in the main experiment are all that are necessary and sufficient to establish that observed changes are selective and specific to the repetitive stimulus, i.e., habitatory.

It should be noted that while our criteria allowed us to conclude that a change was habitatory, they did *not* allow us to conclude that a change that *failed* to meet the criteria was not habitatory. For example, when there was complete generalization in both parts of the experiment, the decreases may well have been habitatory; however, we simply could not be certain. Thus, while our experiments provided evidence of habitatory EP decrements, they were not "strong inference" experiments (Platt 1964).

The point we wish to emphasize is that to establish that any response decrement is habitatory, certain types of controls are required. These are the types of controls found in other learning experiments. For example, classical conditioning studies must include controls to demonstrate that the procedure has not resulted in some general change in state that produces results similar to "real" conditioning, i.e., pseudoconditioning or sensitization. Precisely the same types of controls for establishing the selectivity and specificity of response changes are required in habituation experiments. This is important to note because one of the appealing aspects of habituation experiments has been their apparent simplicity. However, as complex controls are required, this simplicity is only apparent. The importance of habituation experiments derives not from simplicity, but from the ubiquity and

necessity of selective reductions in responses to particular stimuli.

Summary

The goal of the present experiment was to rule out the hypothesis that evoked potential (EP) decrements during repetitive stimulation are due to a change in the subject's state; i.e., that the decrements are part of a general, non-selective, non-specific decrement in all EPs as a result of a change in state during the course of the experiment. To this end, we obtained average evoked potentials (AEPs) to tone pips of two different frequencies; pips of one frequency were the repetitive stimuli, and pips of the other frequency served as "test" stimuli. Before and again after a 15 min series of repetitive pips, AEPs to the repetitive stimuli and to the test stimuli were obtained. We found that from the beginning to the end of the 15 min repetitive-pip series, certain components of the AEPs to the repetitive stimuli decreased significantly in amplitude. However, comparable components in the AEPs to the test stimuli did not demonstrate significant decrements. These results suggested that the decrements were *not* part of a non-selective, non-specific, state-related decrement in all AEPs. However, the selective decrements could have been attributable to either of two aspects of the repetitive tone pips; their repetitive aspect, or their frequency. Because there were two independent variables, a counterbalanced design was required. We found that *regardless of which frequency was repetitive*, there were significant decrements in the AEPs to the repetitive stimuli and smaller decrements (if any) in the AEPs to the test stimuli. This ruled out the frequencies of the tone pips as critical factors in the decrements; by the process of elimination the repetitive aspect of the stimuli remained as the critical variable. Only decrements that were specific to the repetitive stimulus were considered to be habituatory.

All of the habituatory decrements were in components subsequent to the initial AEP

components and had latencies in the range of 17–105 msec. In several cases, when subjects participated in a supplemental experiment, the same AEP components demonstrated habituatory decrements in both experiments.

Résumé

Diminutions d'amplitude du potentiel évoqué dans le cortex auditif. II. Test critique d'habituation

Le but de cette expérience est d'infirmier l'hypothèse suivant laquelle les diminutions d'amplitude du potentiel évoqué (EP) au cours de stimulations répétitives sont dûes à une modification de l'état du sujet, c'est-à-dire qu'elles font partie d'une diminution générale non sélective, non spécifique dans tous les EPs, résultant d'une modification d'état au cours de l'expérience. A cette fin, nous avons obtenu des potentiels évoqués moyens (AEPs) à des pips sonores de 2 fréquences différentes: les pips de l'une de ces fréquences constituaient des stimuli répétitifs et les pips de l'autre fréquence servaient de stimuli-tests. Les AEPs aux stimuli répétitifs et aux stimuli-tests sont obtenus avant et à nouveau après une série de pips répétitifs, de 15 minutes. Nous observons que du début à la fin de la série de 15 minutes de pips répétitifs, certaines composantes des AEPs aux stimuli répétitifs diminuent de façon significative en amplitude. Cependant les composantes comparables des AEPs aux stimuli-tests ne montrent pas de diminution significative. Ces résultats suggèrent que les diminutions d'amplitude ne font pas partie d'une diminution liée à l'état non sélectif et non spécifique de tous les AEPs. Cependant les diminutions sélectives pourraient être attribuables à n'importe lequel des deux aspects des pips sonores répétitifs, leur aspect répétitif ou leur fréquence. Du fait qu'il s'agit là de deux variables indépendantes, un protocole expérimental contrebalancé s'est avéré nécessaire. Nous avons trouvé qu'indifféremment de la fréquence répétitive, il y avait

des diminutions significatives des AEPs aux stimuli répétitifs et des diminutions plus faibles ou nulles des AEPs aux stimuli-tests. Ceci élimine l'hypothèse que les fréquences des pips sonores soient un facteur critique de ces diminutions d'amplitude; du fait de ce processus d'élimination, l'aspect répétitif des stimuli demeure une variable critique. Seules les diminutions d'amplitude spécifiques aux stimuli répétitifs ont été considérées liées à l'habituation. Toutes les diminutions par habituation affectent les composantes consécutives aux composantes initiales des AEPs et ont des latences situées dans la gamme de 17–105 msec. Dans plusieurs cas, lorsque les sujets ont participé à une expérience supplémentaire, les mêmes composantes AEPs ont montrés des diminutions d'habituation dans les deux expériences.

We wish to acknowledge the invaluable secretarial assistance of Elaine Hackelman, and technical assistance for the computer analyses of Gary Paige.

References

- Feller, W. An introduction to probability theory and its applications. Wiley, New York, 1968.
- Groves, P.M. and Thompson, R.F. Habituation: a dual process theory. *Psychol. Rev.*, 1970, 77: 419–450.
- Hinde, R.A. Behavioral habituation. In G. Horn and R.A. Hinde (Eds.), *Short-term changes in neural activity and behavior*. The University Press, Cambridge, 1970: 3–40.
- Horvath, R.S. Variability of cortical auditory response. *J. Neurophysiol.*, 1969, 32: 1056–1063.
- Key, B.J. Correlation of behaviour with changes in amplitude of cortical potentials evoked during habituation by auditory stimuli. *Nature (Lond.)*, 1965, 207: 441–442.
- Petrek, J., Golda, V. and Lisonek, P. Changes in cortical acoustic responses in cats during long-lasting rhythmic stimulation. *Activ. nerv. sup. (Praha)*, 1967, 9: 284–286.
- Petrek, J., Golda, V. and Lisonek, P. Cortical response amplitude changes produced by rhythmic acoustic stimulation in cats. *Exp. Brain Res.*, 1968, 6: 19–31.
- Platt, J.R. Strong inference. *Science*, 1964, 146: 347–353.
- Siegel, S. *Nonparametric statistics for the behavioral sciences*. McGraw-Hill, New York, 1956.
- Sommer-Smith, T.A. and Morocutti, C. Cortical and subcortical evoked potentials during conditioning. *Electroenceph. clin. Neurophysiol.*, 1970, 29: 383–391.
- Teas, D.C. and Kiang, N.Y.-s. Evoked responses from the auditory cortex. *Exp. Neurol.*, 1964, 10: 91–119.
- Thompson, R.F. and Spencer, W.A. Habituation: A model phenomenon for the study of neuronal substrates of behavior. *Psychol. Rev.*, 1966, 73: 16–43.
- Westenberg, I.S., Paige, G., Golub, B. and Weinberger, N.M. Evoked potential decrements in auditory cortex. I. Discrete-trial and continual stimulation. *Electroenceph. clin. Neurophysiol.*, 1976, 40: 337–355.