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A. ACOUSTICALLY EVOKED CORTICAL POTENTIALS IN THE RAT DURING SLEEP AND WAKING

It is now clear that cortical potentials evoked by sensory stimulation are not the same in sleep as in waking. Amplitudes of cortical evoked potentials change with the onset of slow-wave sleep and undergo other changes during sleep marked by rapid eye movements and a low-voltage fast electrocorticogram. Our interest in these changes stemmed from studies of behavioral and pharmacological influences on acoustically evoked potentials in the rat. From the standpoints of experimental control and the interpretation of data it seemed important to know how the potentials might be modified by the behavioral and central changes associated with natural sleep.

Evoked potentials recorded from the primary auditory cortex of cats during sleep and waking have been described in many reports,[5-7, 10, 14, 18-21, 27] but we know of no similar data for the rat. Moreover, the data from these studies of cortical evoked potentials in the cat during sleep and waking are marked by a number of inconsistencies. Some possible reasons for these inconsistencies seemed fairly apparent: differences in the amount of stimulus control, inadequate sampling procedures, difficulties in determining the "state" of the organism, and a rather singular failure in some instances to treat evoked potentials in a reasonably quantitative fashion. The present work was undertaken to determine how click-evoked cortical potentials in the rat change as a function of sleep. We hoped that some of the shortcomings in the earlier work with cats might be avoided.

1. Methods

Ten Sprague-Dawely male albino rats were used. Electrodes were permanently implanted in the auditory cortex of both hemispheres and in various subcortical structures. All electrodes were made from stainless steel, teflon-insulated wire, 125 μ in diameter. Cortical electrodes were bipolar or tripolar arrangements with a ball-tipped pole on the dural surface. The exposed tip of the deep pole (s) was an etched point, 0.5 mm long, placed in different animals at a depth of 1.0 mm, 1.5 mm or 2.5 mm. Hippocampal activity was recorded from similar deep electrodes, while larger wires laid against the occipital bone served in recording neck muscle activity. All evoked potentials described in this report are from cortical "monopolar" recordings. An electrode over the olfactory bulbs or over the cortex just posterior to them served as the reference.

At 6:00 p.m. on the day before an experiment the subject was put in a motor-driven wheel that moved just fast enough to keep the animal awake. He was taken from the wheel at noontime of the experimental day, put in his home cage and allowed to eat, drink, and groom. He was then put in a restraining device[16] and placed in

a sound-attenuating chamber. The restraining device, to which the subjects were adapted for 4 or 5 days, was largely a matter of convenience, but the constraints it placed on movement of the head and body were sufficient to eliminate all but very minor fluctuations in stimulus intensity. The acoustic stimuli were clicks generated by 4.0-V, 0.1-msec pulses applied to a Model 11 KLH speaker located 94 cm above the subjects. They were presented against a wideband low-level masking noise (0.05 V rms) at 1/sec throughout each session. The rats were exposed to these stimuli for at least 4 sessions before any data were taken. With one exception data were obtained from at least 4 experimental sessions with each rat. Sessions were at least 110 min long.

Potentials were amplified by Grass P511 AC preamplifiers and recorded on 7-channel FM magnetic tape and on a Grass Model III EEG. Details of the implanting and recording procedures have been described previously.[17]

Sliding (or moving) averages were obtained with the aid of a PDP-4 computer (Digital Equipment Corporation). Sixty evoked potentials were included in each average. Instead of averaging successive blocks of 60 responses, the computer advanced in steps of only 12 responses so that the second average, for example, contained 48 of the evoked potentials included in the first average. We have felt justified in using average responses computed over periods as long as a minute for the following reasons: (i) The periods in which the animals remained in essentially the same "state" were frequently much longer than a minute. (ii) The waveforms of the cortical evoked potentials were very similar in the several stages of sleep and waking, so that all changes could be regarded mainly as changes in amplitude. (iii) Although transient changes might be lost in the average responses, the advantages of "smoothing" that averaging affords seemed to outweigh this deficiency.

The PDP-4 was also programmed to measure the amplitude and latency of any peak in the average evoked responses, or the amplitude at any fixed time after the stimulus. All amplitudes were measured from a "zero" voltage reference obtained by dividing the average response (obtained from a completely AC coupled system) by a very large number. Particular measures of amplitude and latency were computed for a series of 500 averages, that is, for a period of 101 minutes, and were plotted as a function of time for each sleep session on an X-Y plotter.

2. Results

The predominant "state" of a subject in successive 30-sec periods was determined from the EEG records according to conventional criteria that are illustrated in Fig. XXII-1. In nearly all cases the state classifications were made before the evoked potential data were processed. Our classifications included two states not shown in Fig. XXII-1: an aroused state marked by hippocampal theta rhythms and, frequently, a great deal of movement; a drowsy state with cortical spindling, intermittent slow waves
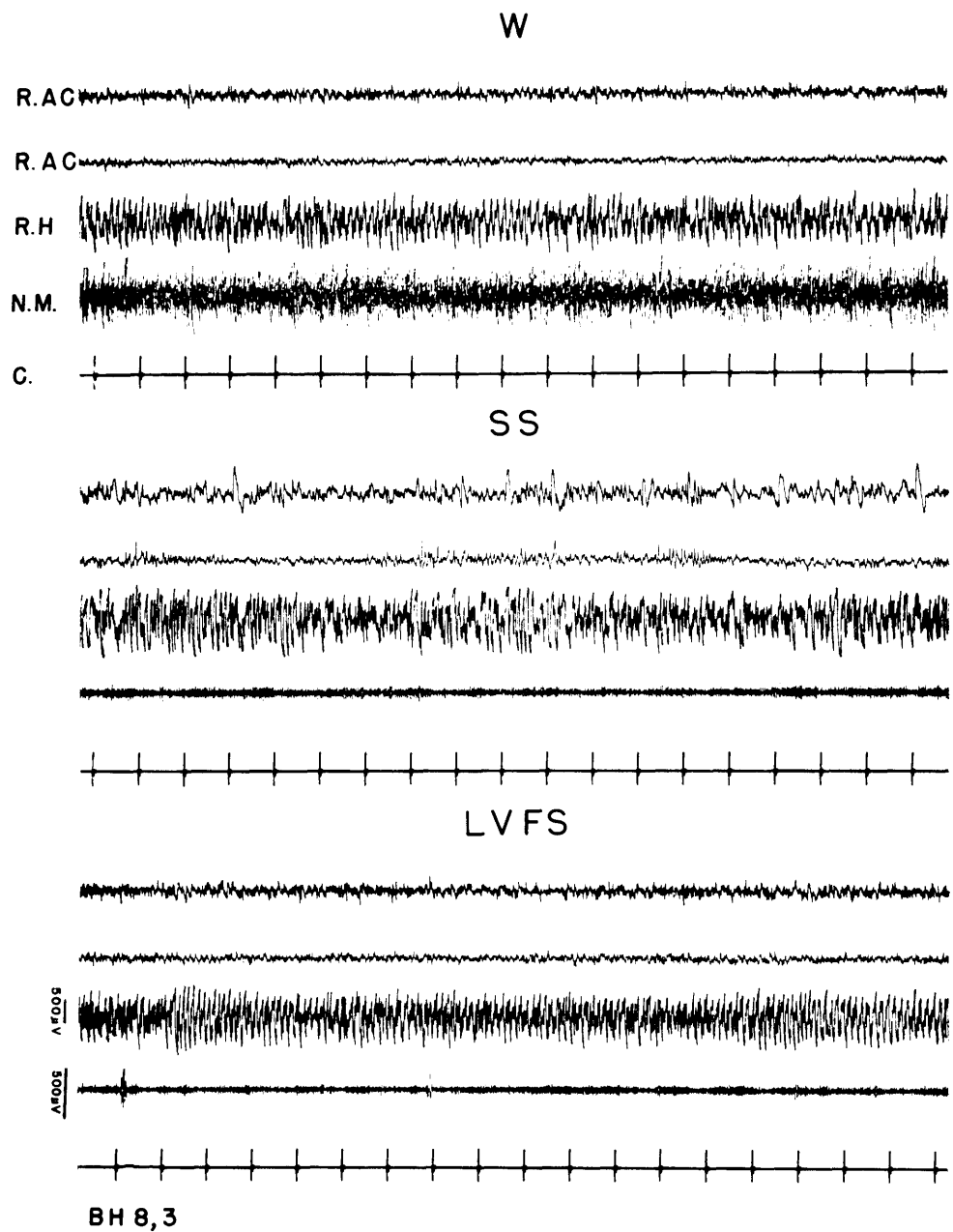
Fig. XXII-1. Electrographic characteristics of 3 state classifications.
R. AC, right auditory cortex (upper trace from transcor-
tical bipolar electrode; second trace from monopolar sur-
face electrode); R.H, right hippocampus; NM, neck muscle;
C, clicks presented at 1/sec. Waking (W) marked by low-
voltage fast cortical activity, some hippocampal theta
waves, and high neck muscle potentials. Slow-wave sleep
(SS) has slow high-voltage cortical and hippocampal activ-
ity and low neck muscle activity. Low-voltage fast sleep
(LVFS) has low-voltage fast cortical pattern, regular hip-
pocampal theta activity and low muscle potentials showing
occasional twitches. Upper amplitude calibration applies
to all signals except neck muscle.

and neck muscle activity intermediate to that in waking (W) and slow-wave sleep (SS). We have included the aroused state in the W periods for the purposes of this presentation, since it occurred only rarely, and we have included the drowsy state in the SS periods. Click-evoked cortical potentials recorded during drowsy and SS periods were essentially alike.

The clearest changes in click-evoked cortical responses were seen in potentials recorded from a depth of 1.0-1.5 mm in the cortex. Examples of these responses are shown in Fig. XXII-2 for 4 of the 5 subjects who had at least one electrode at this depth.
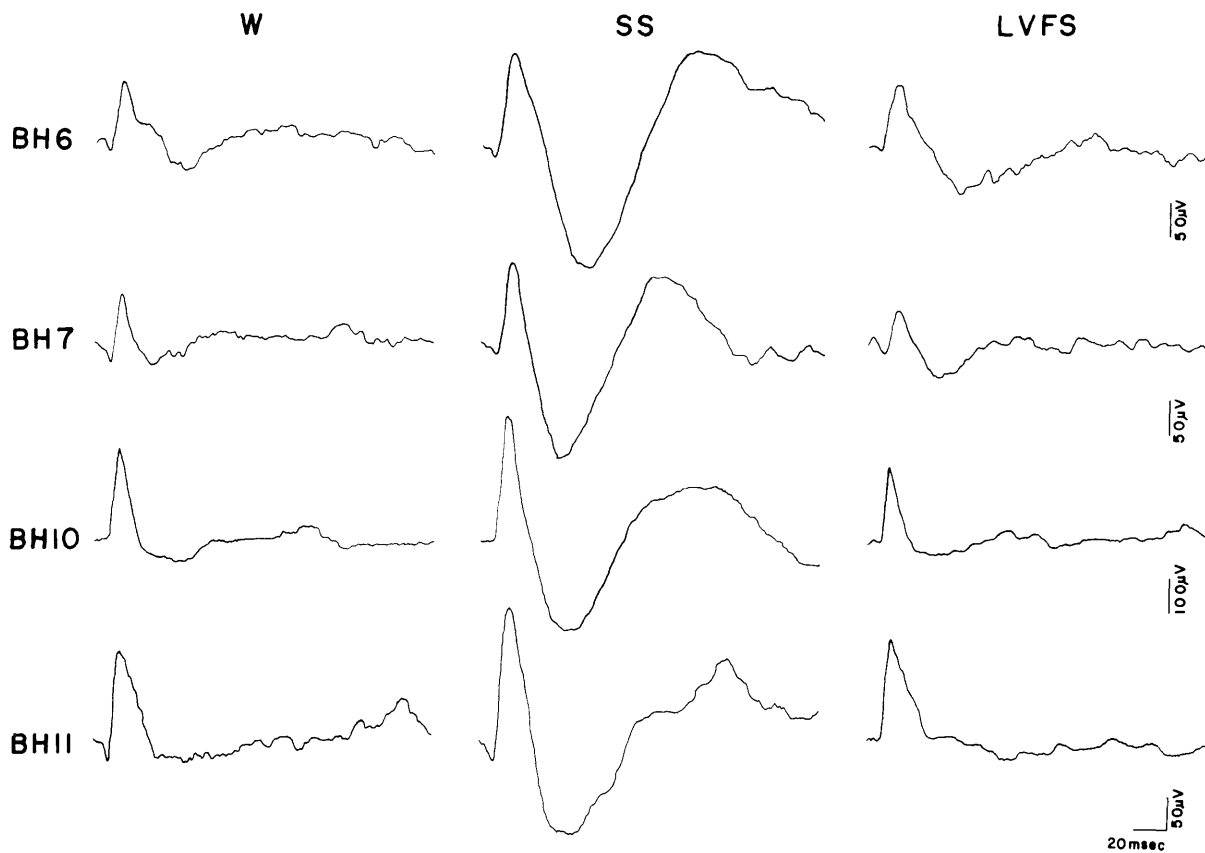
Fig. XXII-2. Average click-evoked responses from 4 subjects (BH6, BH7, BH10, BH11) recorded at depths of 1.0-1.5 mm during W, SS, and LVFS. In this and all succeeding figures positive changes of potentials are indicated by downward deflections.

These average responses showed a marked increase in the amplitudes of all components during SS, especially in the late positive and negative waves which were quite small during W and low-voltage fast sleep (LVFS). In general, the evoked responses recorded

during W and LVFS were very similar. This can be seen in Fig. XXII-3 where amplitudes of sliding averages at 3 fixed times after the stimulus have been plotted. The

Fig. XXII-3. Mean amplitudes of evoked responses recorded at 1.0-1.5 mm depth at 3 fixed times (20.8, 59.2, 140 msec) following stimulus onset. Times at which potentials were mea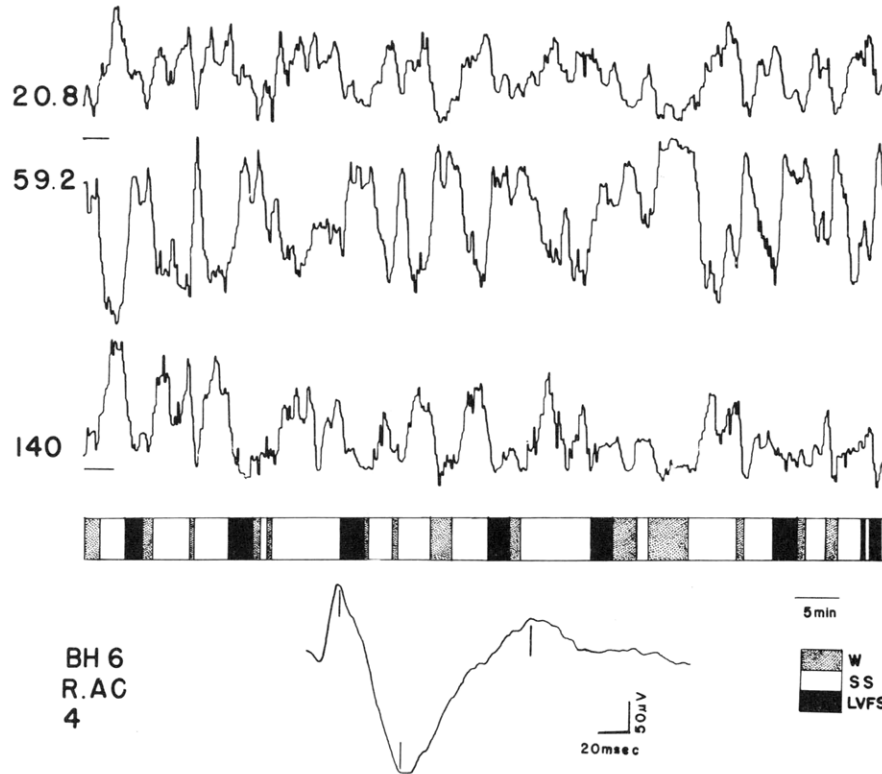sured are indicated by vertical lines in average response shown at bottom. Amplitudes computed for 500 sliding averages covering 101 min. Changes in state indicated by ribbon beneath curves. Zero voltage references indicated by short horizontal lines at left; top one applies to two upper curves.

data are from the first 101 min of a single sleep session for one subject. The times at which the potentials were measured, 20.8, 59.2, and 140 msec, are indicated by the vertical lines on the average response shown at the bottom of the figure. The state of the subject throughout this period is indicated by the ribbon below the amplitude plots. It is clear that all three waves in these deep cortical responses showed marked increases in amplitude during SS, while the potentials recorded during W and LVFS were not appreciably different. The obvious negative correlation between the middle plot and the other two reflects the fact that the middle one is for a positive-going component, while the other two are measures of negative waves. All three waves

grow larger in SS.

Potentials recorded from the cortical surface exhibited one change that seemed to reflect the changes in deep potentials shown in Figs. XXII-2 and XXII-3. A late surface-negative wave with a peak at approximately 80 msec increased significantly during SS. This can be seen in Fig. XXII-4 where the amplitudes of both surface and deep potentials have been plotted for another subject. The surface response was measured at
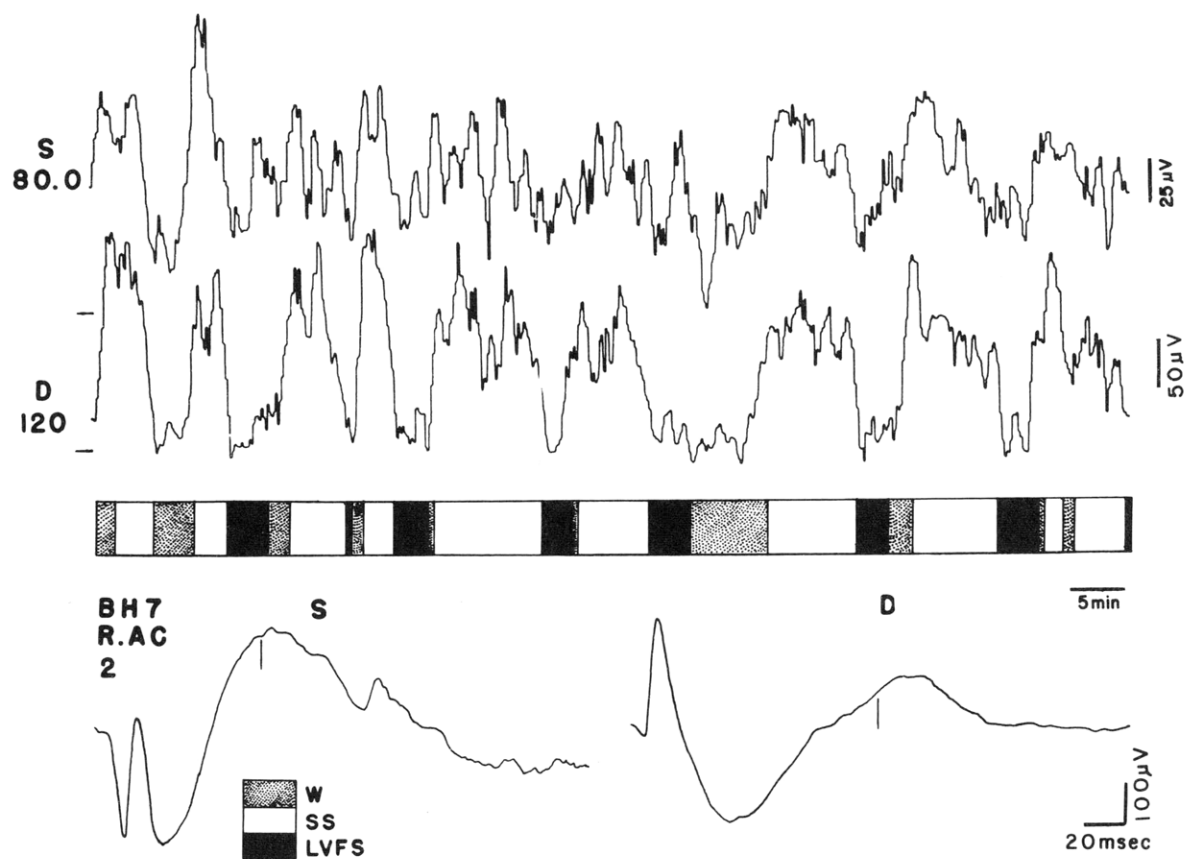
Fig. XXII-4. Amplitudes of late deflections computed from sliding averages of click-evoked responses recorded simultaneously from surface (S) and deep (D) electrodes. Surface response measured at 80 msec; deep response, recorded at depth of 1.0-1.5 mm, measured at 120 msec. Zero reference voltages indicated by short horizontal lines at left. State changes indicated by ribbon. Vertical lines below average responses show times at which measurements were made.

80 msec where the changes in SS generally appeared largest. The deep response was measured at 120 msec when it was also negative, to show the correlation between the amplitudes of deep and surface waves. The deep response was not measured at 80 msec

because the changes were rather small at this time, 80 msec being roughly the transition point between the late positive and negative waves. But as noted above, changes in all three waves of the deep responses were highly correlated, so that the relation would be the same if the deep response were measured at any time between 20 msec and 140 msec. The correlation between the two curves is fairly obvious, though not entirely consistent
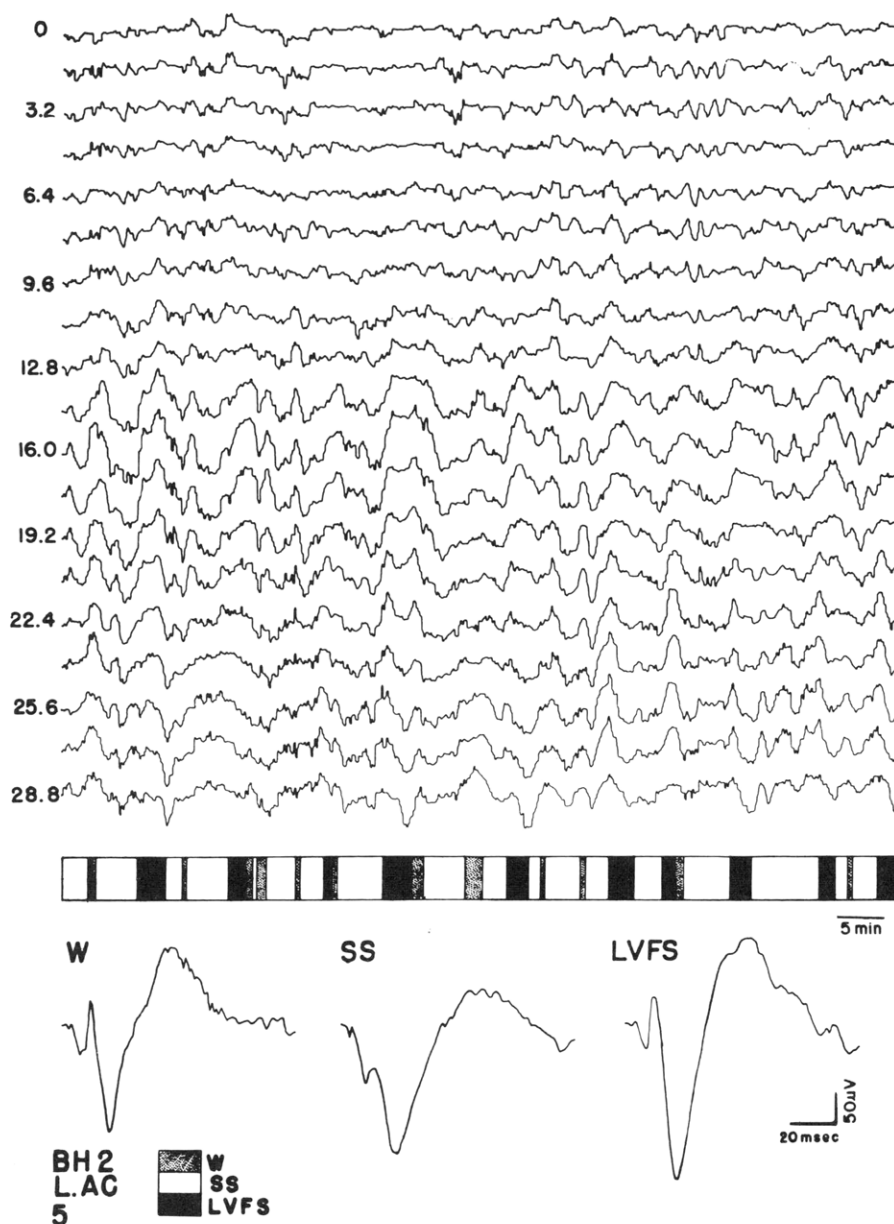

Fig. XXII-5. Amplitudes of sliding averages of click-evoked responses measured at 1.6 msec intervals from 0 to 28.8 msec. Potentials recorded from surface electrode. Characteristic waveforms shown for 3 states at bottom. States indicated as in previous figures.

throughout the session. Agreement between response amplitudes and state was generally better for the deep responses than for the late surface-negative wave.

Early components of the cortical surface response exhibited changes which are illustrated in the average evoked responses shown at the bottom of Fig. XXII-5. During SS the initial positive wave became larger and peaked several msec later than in W or LVFS. The first negative wave in the W potentials also changed in the positive direction during SS, and frequently appeared as only a negative-going deflection on a positive wave. At the top of Fig. XXII-5 is a series of amplitude measurements that were made at 1.6 msec intervals from the time of stimulus onset at 0 to 28.8 msec. Notice the pattern of amplitude changes that first emerges in these plots at 12.8-13.4 msec. During SS the changes are in the positive (downward) direction, while they are in the negative direction during W and LVFS. This pattern of changes is most conspicuous at approximately 16 msec, but it is present throughout the period occupied by both early components, as though, perhaps, one process — not two — were modified by sleep and waking. Of significance also is the finding that amplitudes of the early components were not appreciably different in W and LVFS periods.

In Fig. XXII-5 it can be seen that the pattern of amplitude changes associated with the early components of the cortical response gradually evolves into another pattern beginning at approximately 20.8 msec. This pattern reflects the changes in the second positive wave, which is seen to be especially large in the average response taken from a LVFS period. Careful examination of the amplitude changes at 28.8 msec, for example, will reveal that within most, but not all, LVFS periods there was an increase in the amplitude of this second positive wave which peaked at approximately 30 msec. This increase in amplitude, though not a consistent feature of all LVFS periods, occurred with sufficient frequency that we believe it represents a genuine physiological change. Additional examples of this change are shown for other subjects in Fig. XXII-6. The successive, nonoverlapping averages presented in this figure were obtained from individual LVFS periods of at least 3-min duration.

It can be seen in some responses in Fig. XXII-6 that the increases in the second surface-positive wave are accompanied by a similar increase in the late negative wave that follows it. This negative wave of relatively short duration peaked at approximately 60 msec. It appeared to be somewhat distinct from the slower negative wave that peaked at 80 msec and was seen to grow during SS. A clear example of the difference can be seen in the average responses shown in Fig. XXII-7. It is not clear whether this difference represents a change in a single process or whether the two waves are related to different generators. Amplitudes of the two waves were in part complementary in that the later wave was larger in SS and relatively small during LVFS, while the earlier one exhibited the opposite trend. This can be seen in the peak latency plot of Fig. XXII-7. To obtain this curve, the computer searched for the largest negative

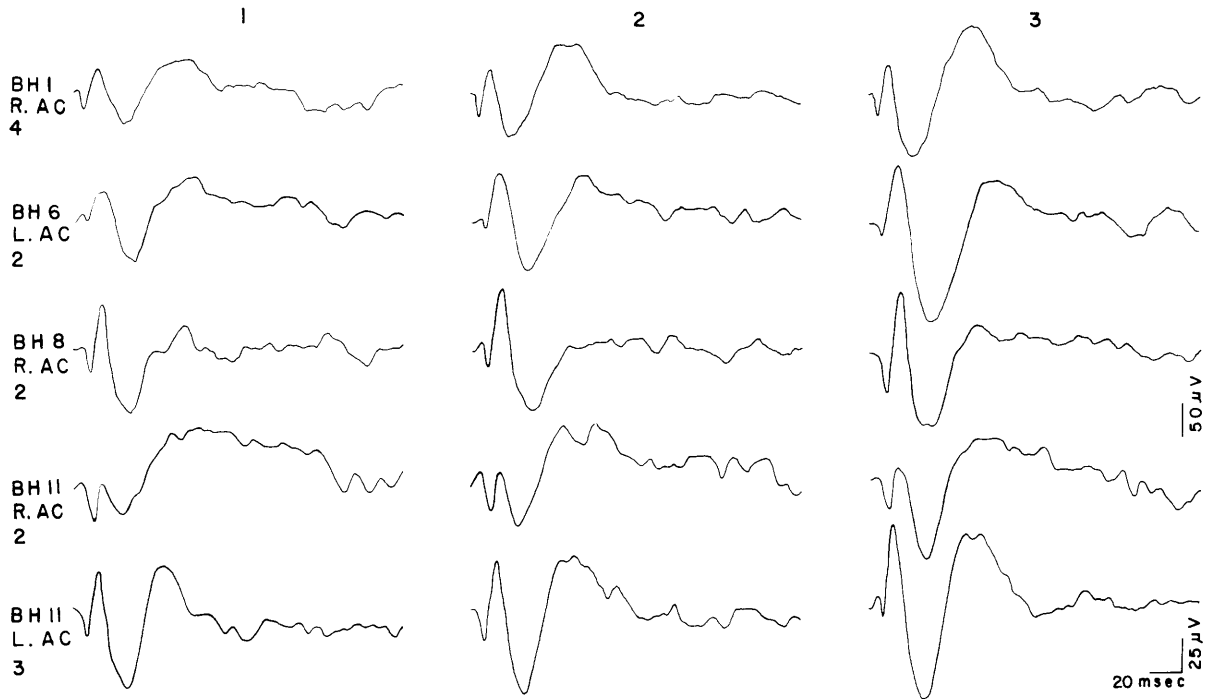Fig. XXII-6. Three successive nonoverlapping averages of click-
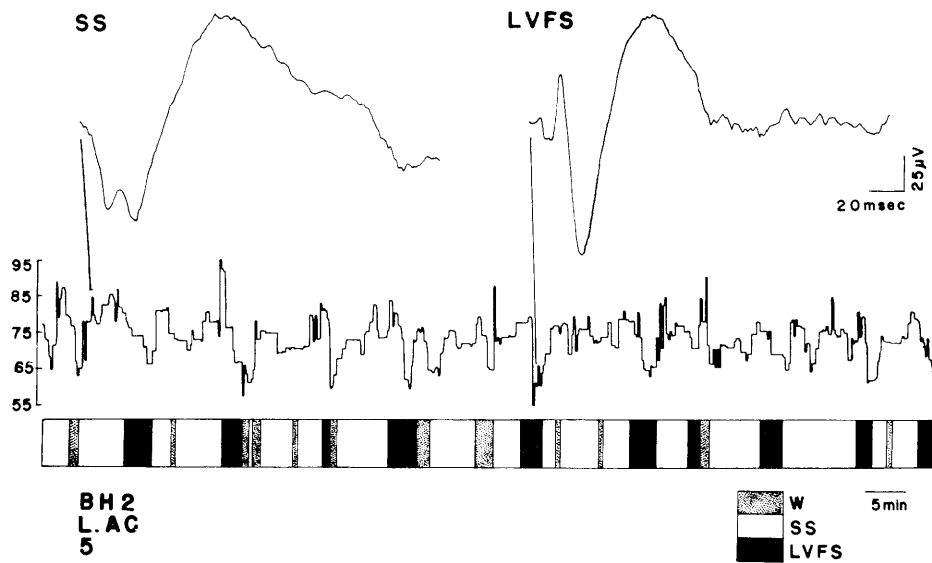evoked surface responses from LVFS episodes for 4
subjects.

Fig. XXII-7. Peak latency of late negativity in sliding averages of click-
evoked surface responses. Typical waveforms from SS and
LVFS periods shown at top. Vertical lines indicate times at
which averages were obtained. Scale at left indicates time
in msec. See text for further details.

voltage between 40 msec and 100 msec in a series of 500 average responses. The times at which this voltage occurred were then plotted as a function of the time at which the evoked potentials were recorded during the session. It is fairly clear that the shortest latencies of the peak negativity occurred during LVFS periods, and the longer latencies are rather consistently associated with SS epochs. The plot is a bit "noisy," largely because in some averages the peaks were not well defined and the largest voltages were located at essentially random times. Minor fluctuations in the waves, even when well defined, add to the jitter.

The changes in click-evoked cortical potentials described above are, on the whole, consistent features of the data, which, however, exhibited some interesting variability. Several forms that this variability may take are illustrated in Fig. XXII-8. The averaged
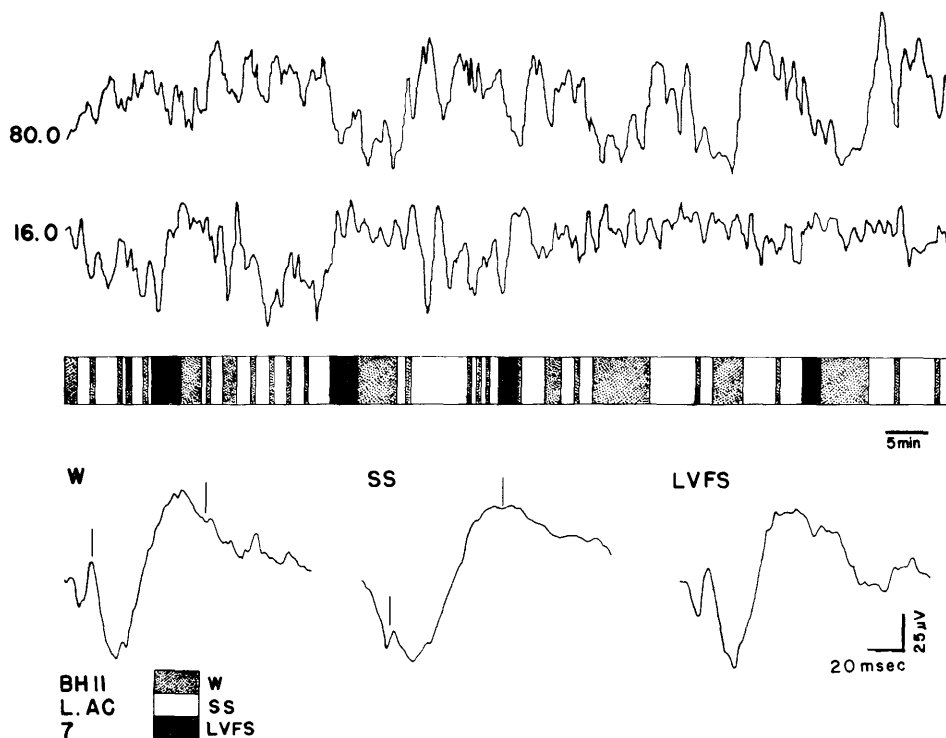
Fig. XXII-8. Amplitudes of sliding averages of surface click-evoked responses at 16.0 and 80.0 msec. Times at which responses measured are indicated by vertical lines above typical average responses at bottom.

surface responses shown at the bottom of the figure are quite typical of those described above. Amplitudes of these waveforms were measured at 16.0 msec, the peak of the primary negative wave during W and LVFS, and at 80.0 msec, the peak of the late negative wave during SS. Of interest are the relatively large swings in amplitude at 16.0 msec

in roughly the first half of the record, and the absence of any comparable changes in the second half of the record. The large changes in amplitude of the negative wave at 80.0 msec conform, by and large, to the usual pattern and indicate that the animal was indeed passing from one state to another in agreement with the EEG criteria. There was no indication in any of the available measures that this washout of the changes at 16.0 msec should be expected. Notice, too, in both amplitude curves that within episodes of one state or another there are occasionally large changes in amplitude that seem quite at odds with the over-all trends.

3. Discussion

We were rather surprised to find such large and consistent changes in the click-evoked potentials recorded from a depth of 1.0-1.5 mm in the cortex. The marked increase in all components of the deep response during SS appeared generally to be less variable than the more complex changes in the surface responses. One part of the surface response, the late negative wave which peaked at approximately 80 msec, seemed to reflect the changes in late components of the deep response, being appreciably larger during SS than it was in W or LVFS. It is probable that the increased positivity during SS in the early part of the surface response reflects to some extent the increased amplitude of the first negative wave in the deep response. Although there are these correlated state-dependent changes in the deep and surface responses, the precise relationships between the deep and surface potentials are still by no means clear.

Average click-evoked responses obtained from periods of LVFS were on the whole very similar to those obtained from the awake animal. Early components of the responses showed no systematic differences, a finding which seems to be in substantial agreement with the published data on acoustically evoked potentials in cat cortex.[7,19,27] Berlucchi et al. have reported[5] an increase in the first negative wave and to a lesser extent in the first positive deflection of click-evoked potentials in cat cortex during rapid eye movements (REM) in LVFS. (Okuma and Fujimori[23] have reported a similar increase within LVFS periods in somatic and visual cortical potentials during REM.) Teas and Kiang[27] have also noted in one of their figures a prominent first negative deflection during LVFS. We have found no evidence in the average responses that the first negative wave of the surface response is consistently larger in LVFS than it is in W, although there were instances when this was so. One case is illustrated in Fig. XXII-6 where the increase in the first negative wave is seen to accompany the increase in the late components during episodes of LVFS. Whether or not the augmented early negative wave seen sometimes in our experiments was related to REM we cannot say.

One very consistent report from studies of evoked potentials during sleep has been that early components of electrically evoked cortical responses recorded from specific

sensory areas are larger in LVFS than they are in both W and SS. In the somatic system this appears to be the case when the electrical stimuli are applied to the skin,[11,12,23] directly to peripheral nerve,[1,3,15,25] to the medial lemniscus[12] or to the ventrobasal nuclei of the thalamus.[2,11-13,23,26] In the visual system electrically evoked cortical responses have been found to be largest during LVFS with stimulation of the optic tract[8,9,13,23] or the lateral geniculate body.[4,9,13,24,26] With stimulation of the brachium of the inferior colliculus auditory cortical responses were also found to be largest in LVFS.[10] The fact that the initial positive wave in the cortical response to "physiological" stimuli, acoustic or photic,[22] does not exhibit the consistent increase in LVFS seen with electrical stimulation suggests a critical interaction between state-dependent changes and the mode of stimulation.

It is clear in the data presented here that significant late components were found in the cortical surface responses recorded during W, SS, and LVFS. A late negativity peaking at approximately 80 msec was seen to increase during SS. This fact is an apparent contradiction of findings reported by Huttenlocher[20] and by Teas and Kiang[27] for click-evoked cortical potentials in the cat. These investigators found a similar late negativity present only in the awake animal. There is ample evidence, however, indicating that late components of evoked potentials to acoustic and somatic stimulation in nonspecific cortical areas exhibit appreciable increases in amplitude during SS.[1,3,15,19,28,29] It may be that similar nonspecific responses can be recorded from the auditory cortex of the rat. It is noteworthy that the largest increases in late evoked activity encountered during SS were recorded from a depth of 1.0-1.5 mm in the cortex. If comparable data were available for the cat cortex, the relationship between click-evoked cortical potentials in the two species might be clarified.

The absence of late cortical responses during LVFS is also characteristic of the earlier data from studies of cat auditory cortex,[5,19,20,27] although Teas and Kiang[27] did record a late positivity that underwent changes in LVFS. On the other hand, a positive wave peaking at approximately 30 msec was prominent during all three states in our results. It sometimes increased during episodes of LVFS, together with a negative wave peaking around 60 msec. A very similar change has been described for somatic evoked potentials recorded from the anterior sigmoid gyrus[1] and the foot of the cruciate sulcus.[15] Guilbaud[15] has shown that the late components of these potentials during LVFS have several properties of lemniscal responses and differ in this respect from the late components which are large in SS. It is interesting that from a single location on rat auditory cortex one can record two forms of late responses that resemble late potentials recorded outside classical projection areas of the cat cortex.

Although many of our measures showed a high correlation with the changes of sleep and wakefulness, it was also clear that other systematic sources of variability affected the evoked potentials, e.g., the reduction of state-dependent changes in early but not late

components illustrated in Fig. XXII-8. The methods of data analysis employed in this study do not permit such changes to go unnoticed so easily, and therefore provide a more complete, though perhaps less simple, picture of the multiple influences on cortical evoked potentials.

A. A. Borbély, R. D. Hall

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B. EFFERENT INHIBITION OF ELECTRICALLY STIMULATED RESPONSE IN CAT AUDITORY-NERVE FIBERS

Recent work by Wiederhold[1] has shown that electrical stimulation of an efferent neural feedback path to the ear, known as the crossed olivocochlear bundle (COCB), can significantly inhibit the response of most auditory-nerve fibers to certain acoustic stimuli. Moxon[2] has shown that application of electric current pulses to electrodes placed on the cochlea can result in spike responses in single auditory-nerve fibers. The present work is a continuation of this research, combining the techniques of electrically stimulating the COCB and the cochlea. This study is concerned with the effect of COCB stimulation on the single-fiber response to electric stimulation of the cochlea. A comparison is made of this effect with that of COCB stimulation on the response to comparable acoustic stimuli.

The single-fiber response to electric pulses and gated sinusoids applied to the cochlea has two components which we shall designate simply components "A" and "B". Stimulation of the COCB has been found to inhibit component B in much the same way as the response to acoustic stimulation is inhibited by COCB stimulation. Component A is not consistently affected by COCB stimulation.

Component A is interpreted as the response of the fiber to direct electric depolarization of the axonal or dendritic membranes central to the fiber's synapses on the hair cells in the cochlea. Figure XXII-9 shows post-stimulus-time histograms showing the effect of COCB stimulation on the responses to electric pulses, acoustic clicks, gated electric sinusoids, and acoustic tone bursts. In these PST histograms the time distribution of spikes is taken relative to the onset of stimulation, either acoustic or electric. In the histograms of responses to electric pulses, the first peak represents response component A, the smaller successive peaks, component B (no A response is seen in Fig. XXII-9C). COCB stimulation has little effect on the A response in Fig. XXII-9A. Note that the shapes of the histograms for both of these acoustic-electric pairs are very similar, and that the effect of the COCB stimulation on response component B is nearly the same as that on the response to the acoustic stimulus in each case. Figure XXII-10 shows level series relating the number of firings to stimulus level for responses to acoustic tone bursts and gated electric sinusoids for one fiber.

Figure XXII-11 shows the effect of COCB stimulation on response component A. Figure XXII-11a shows percentage reduction in component A plotted against fiber characteristic frequency (CF) for 29 fibers from 4 cats. CF is taken as the frequency of acoustic tone burst for which the fiber's threshold of response is lowest. Figure XXII-11b is a histogram of the same data showing the distribution of this effect. These data were obtained from histograms such as those of Fig. XXII-9A.
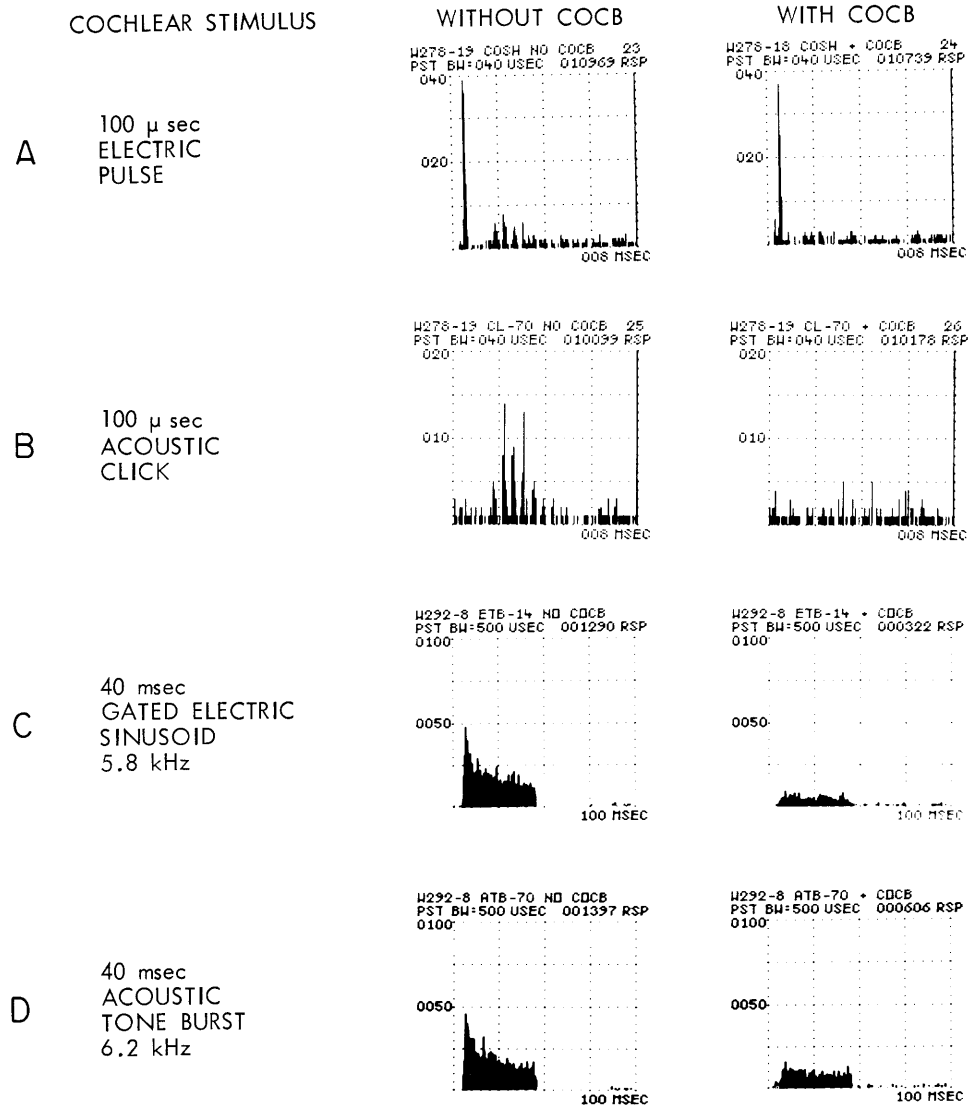
COCHLEAR STIMULUS WITHOUT COCB WITH COCB

H278-19 COSH NO COCB 23
PST BW=040 USEC 010969 RSP
040

020

008 MSEC

H278-18 COSH + COCB 24
PST BW=040 USEC 010739 RSP
040

020

008 MSEC

A 100 μ sec
ELECTRIC
PULSE

H278-19 CL-70 NO COCB 25
PST BW=040 USEC 010099 RSP
020

010

008 MSEC

H278-19 CL-70 + COCB 26
PST BW=040 USEC 010178 RSP
020

010

008 MSEC

B 100 μ sec
ACOUSTIC
CLICK

H292-8 ETB-14 NO COCB
PST BW=500 USEC 001290 RSP
0100

0050

100 MSEC

H292-8 ETB-14 + COCB
PST BW=500 USEC 000322 RSP
0100

0050

100 MSEC

C 40 msec
GATED ELECTRIC
SINUSOID
5.8 kHz

H292-8 ATB-70 NO COCB
PST BW=500 USEC 001397 RSP
0100

0050

100 MSEC

H292-8 ATB-70 + COCB
PST BW=500 USEC 000606 RSP
0100

0050

100 MSEC

D 40 msec
ACOUSTIC
TONE BURST
6.2 kHz

Fig. XXII-9.

A: Effect of COCB stimulation on response to electric pulses applied to the cochlea. First peak on the left is component A, following smaller peaks are component B. COCB stimuli: 32 doublet pulses at 400 per second, preceding the cochlear stimulus by 10 msec for all of Fig. XXII-9. Cochlear pulses: 100 μsec duration; 450 μA baseline to peak. Fiber CF = 2.4 kHz. Histograms of responses to 200 identical stimuli.

B: Effect of COCB stimulation on response to acoustic clicks for the same fiber as A. Click level: 70 dB re 100 V baseline-to-peak microphone voltage (100 μsec duration). Histograms of response to 200 identical stimuli.

C: Effect of COCB stimulation on responses to gated electric sinusoids applied to the cochlea. Gated sinusoid: 40 msec duration, level -14 dB re 1.1 mA peak to peak. Fiber CF = 6.2 kHz. Histograms of response to 200 identical stimuli.

D: Effect of COCB stimulation on responses to acoustic tone bursts for the same fiber as C. Tone burst: 40 msec duration, -70 dB re 200 V peak-to-peak microphone voltage. Histograms of response to 200 identical stimuli.
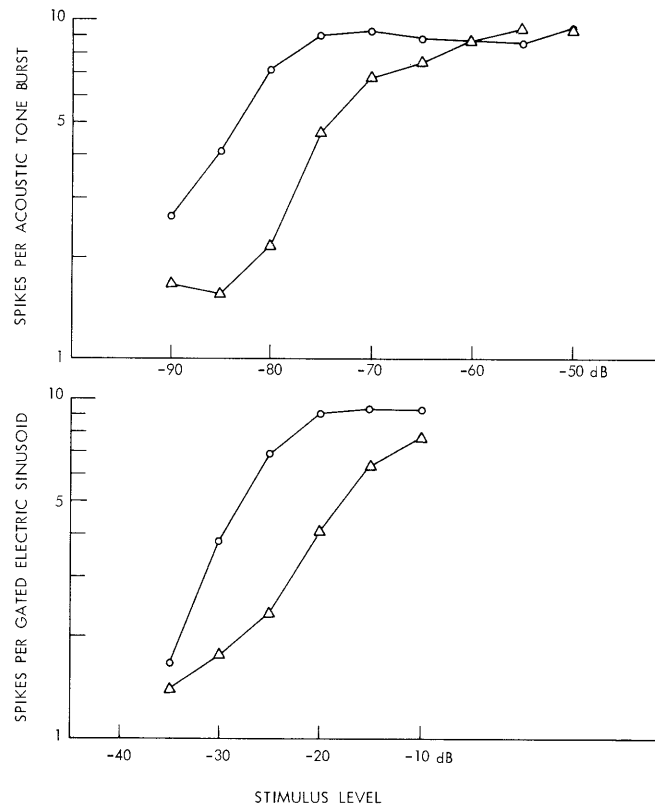
Fig. XXII-10. Comparison of level series curves for response to acoustic tone bursts and gated electric sinusoids with and without COCB stimulation. Fiber CF = 7.9 kHz. COCB stimuli: 32 doublet pulses at 400 per second preceding tone burst or sinusoid by 10 msec. Both tone bursts and sinusoids, 40 msec duration. Acoustic tone burst level re 200 V peak-to-peak microphone voltage. Gated electric sinus-oid level re 1.1 mA peak to peak.

The average effect of COCB stimulation is a 5.7% reduction in response component A. These plots indicate, however, that this reduction varies from fiber to fiber. Cochlear shock stimulus parameters were chosen so that the fiber's probability of responding was between 0.2 and 0.8.

Component B of the response of single fibers to electric stimulation of the cochlea is inhibited significantly by COCB stimulation (e.g., Figs. XXII-9A, XXII-9C, and XXII-10). This response has striking similarities to the response to comparable acoustic stimuli (acoustic clicks are comparable to electric pulses, and acoustic tone bursts to gated electric sinusoids). It is interpreted as resulting from an electromechanical interaction.

The similar effect of COCB stimulation on responses to acoustic stimuli and
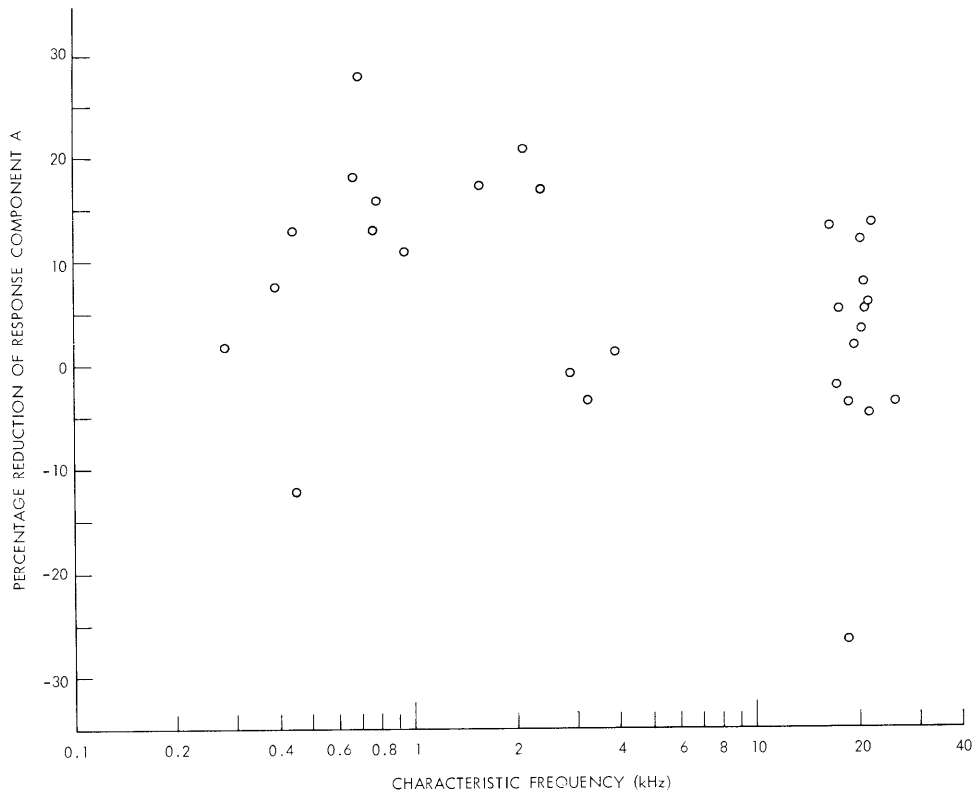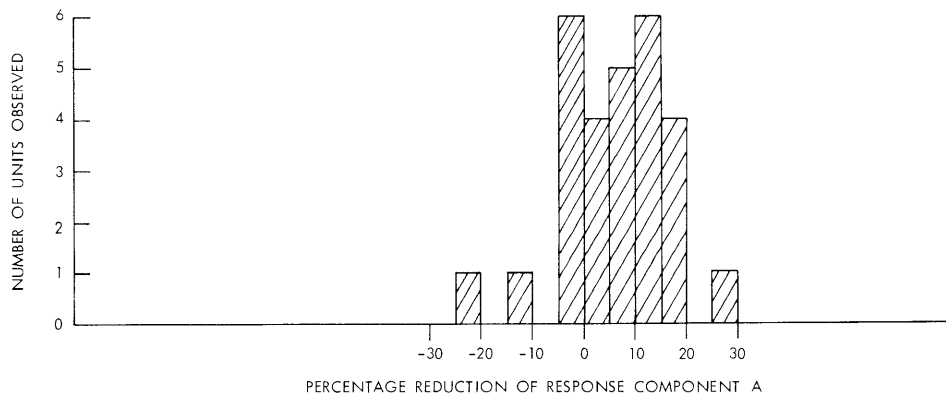
Fig. XXII-11.

(a) Scatter plot of percentage reduction of response component A by COCB stimulation for 29 fibers from 4 different cats. COCB stimuli: 32 doublet pulses at 400 per second, preceding the cochlear stimulus by 10 msec. Cochlear pulses: 100 μsec duration; amplitude adjusted to give fiber firing probability between 0.2 and 0.8. Data points taken from histograms of response to 200 identical stimuli.

(b) Histogram of same percentage reduction data as in (a). Horizontal scale in units of 5% reduction of response component A by COCB stimulation. COCB stimuli same as in (a).

the B component of responses to electrical stimulation of the cochlea suggests that the B response arises from the same excitatory processes as the response to acoustic stimuli. The site of action of these electric stimuli would then appear to be twofold. The B response could be thought of as being initiated peripheral to the endings of the COCB on hair cells and/or afferent dendrites. The A response is unaffected and thus would arise from stimulation central to the COCB endings. This is consistent with a previous interpretation of the origin of the A and B responses.[2]

A full description of this work is presented in S. C. Chamberlain's Bachelor's thesis submitted to the Department of Electrical Engineering, M.I.T., May 17, 1968.

S. C. Chamberlain, E. C. Moxon, M. L. Wiederhold

References

1. M. L. Wiederhold, "A Study of Efferent Inhibition of Auditory Nerve Activity," Ph.D. Thesis, Department of Electrical Engineering, M.I.T., 1967.

2. E. C. Moxon, "Electric Stimulation of the Cat's Cochlea: A Study of Discharge Rates in Single Auditory Nerve Fibers," S.M. Thesis, Department of Electrical Engineering, M.I.T., 1967.

C. AUDITORY NERVE RESPONSES TO ELECTRIC STIMULI

Responses of single fibers in the auditory nerve of anesthetized cats were studied with electric current pulses delivered to the cochlea. Characteristics of these responses were compared with fiber responses to acoustic stimuli. The primary objective was to study the maximum firing rate (number of spikes per unit time) to which a fiber could be driven for both acoustic and electric stimuli.

The purposes of this report are to describe the responses of the auditory nerve to the electric stimuli employed, and to summarize the experimental findings relating to the maximum firing rate.

1. Methods

The preparation has been described by Kiang.[1] Equipment for generating acoustic stimuli and methods for processing single unit data were also the same. In the present experiments equipment was included for generating electric stimuli and for reducing the effects of electric stimulus artifact recorded by the microelectrode in the nerve.[2]

Electric stimuli were usually 100-μsec rectangular pulses (shocks) of amplitudes up to a few milliamperes. Three stimulating electrode configurations were used to deliver electric stimuli to the cochlea and auditory nerve.

In the first configuration a pair of insulated wires exposed at the tips were introduced through the round window and positioned across the basilar membrane in the basal turn of the cochlea, one wire in scala media and the other in scala tympani. A

second configuration consisted of a pair of wires threaded through the shank of a hypo-dermic needle and exposed at the tip. This second assembly could be introduced through the round window and forced through the bone to contact the auditory nerve in the modi-olus. In the third electrode configuration, one of a pair of wires was placed on the intact round window, the other on the surface of the cochlea more apically. Electrode material in all cases was stainless steel.

2. Properties of Responses to Electric Stimuli

Current pulses delivered with all three electrode configurations were capable of evoking responses in auditory nerve fibers. In experiments in which pulses were applied to electrodes across the basilar membrane, however, only a fraction of the units encoun-tered responded even at the highest current levels used (2-3 mA). Indications are that currents injected with these electrodes resulted in high current densities in
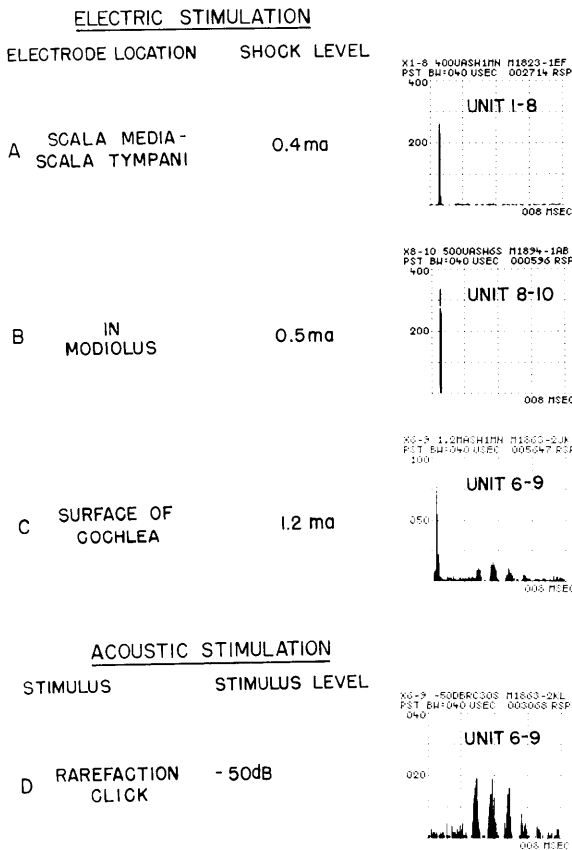
Fig. XXII-12.

PST histograms computed from unit responses to electric shocks and clicks. The characteristic frequency of unit 6-9 was 1.14 kHz. In D, click amplitude is given in terms of electric input to the earphone, −50 dB re 100 V, which is roughly 65 dB Sound Pressure Level.

a small region of the cochlea and that only units located in the vicinity of the electrodes were affected. In contrast, in experiments with stimulating elec-trodes in the modiolus or on the surface of the cochlea, all units encountered

could be stimulated electrically.

The latency of responses to electric stimuli applied to each of the three electrode configurations is illustrated in the post stimulus time (PST) histograms in Fig. XXII-12. The first large peak in the histograms occurs roughly at 0.5 msec in Fig. XXII-12A, XXII-12B, and XXII-12C. This latency is approximately 1 msec shorter than the shortest primary unit latency in response to an acoustic click. The histogram in Fig. XXII-12C shows some smaller, later peaks not present in Fig. XXII-12A and XXII-12B. The click
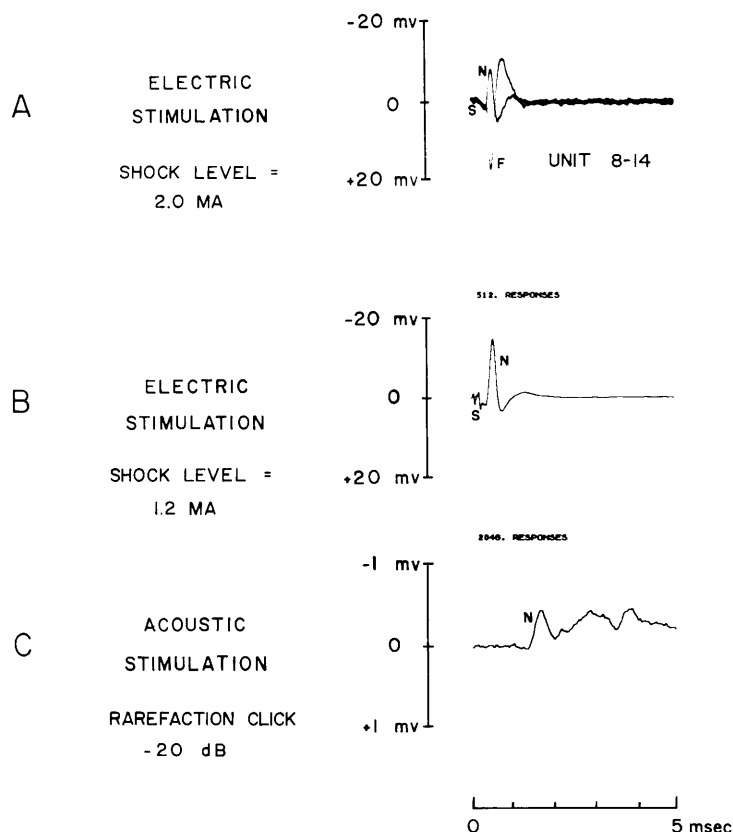

Fig. XXII-13. Potentials recorded by microelectrode in the nerve.

A: Two oscilloscope traces superimposed. Shock level was just at threshold for the single fiber, which responded to only a fraction of the stimuli. In one trace only the whole-nerve response (N) and the stimulus artifact (S) appear. The other has a single fiber spike response (F). Stimulating electrodes were in modiolus.

B: Averaged response to shocks, showing stimulus artifact (S) and whole-nerve response (N). Stimulating electrodes were on surface of cochlea.

C: Averaged response to high-level clicks recorded by the same microelectrode as in B. N, neural response. Click amplitude re 100 V into earphone; roughly 95 dB SPL. Note difference in scale between B and C.

response of the same unit is shown in Fig. XXII-12D. Since it has been suggested that temporal patterns of unit click responses are closely related to mechanical action in the cochlea,[1] the similarities in histograms XXII-12C and XXII-12D suggest that a detectable electromechanical interaction can accompany the current pulse.

The absence of any later peaks in histograms XXII-12A and XXII-12B is probably because of the poor condition of the cochlea in these experiments. Severe physical damage to the cochlea occurred when electrodes were forced into the modiolus, and detrimental chemical reactions apparently occurred with electrodes in the cochlear fluids across the basilar membrane. In both cases the functioning of the cochlea, judged from its electrical activity, was severely disrupted.

With electrodes on the surface of the cochlea, however, no impairment of cochlear function was detectable. Also, with these electrodes unit responses to electric stimuli were not found to differ significantly from responses recorded with electrodes in the modiolus, when nerve fibers were presumably stimulated directly. For these reasons, most of the data in this study were obtained with the electrodes on the surface of the cochlea.

With electric stimulation the microelectrode in the auditory nerve recorded a compound, whole nerve action potential in addition to all-or-none single-fiber action potentials. This compound potential is shown in Fig. XXII-13A (labeled N), along with a fiber spike potential (F) and stimulus artifact (S). Another example of this compound potential is shown in Fig. XXII-12B, with the much smaller compound potential recorded by the same microelectrode in response to a high-level click shown for comparison in Fig. XXII-13C. Because of the larger amplitude of the response to the current pulse, it might be concluded that a relatively large number of auditory-nerve fibers are made to fire synchronously in this situation. The finding that all single fibers encountered responded to electric stimuli and exhibited roughly the same latency would support such a conclusion.

3. Response Rates

Current pulses delivered at sufficiently high repetition rates were able to evoke unit discharge rates substantially higher than the highest rates ever obtained with acoustic stimuli. This result is demonstrated in Fig. XXII-14, in which unit discharge rate is plotted against stimulus level for 3 stimuli: tone at the characteristic frequency (CF), 100-μsec clicks at 1000/sec, and 100-μsec current pulses at 1000/sec. With any of these stimuli the effect of increasing stimulus level is generally to increase a fiber's discharge rate above the spontaneous level, but with the electric stimuli discharge rate grows more rapidly with stimulus level and reaches values not attained with the highest click or tone levels.

The highest spike rates obtained in the cat's auditory nerve rarely exceed 200/sec.[1]
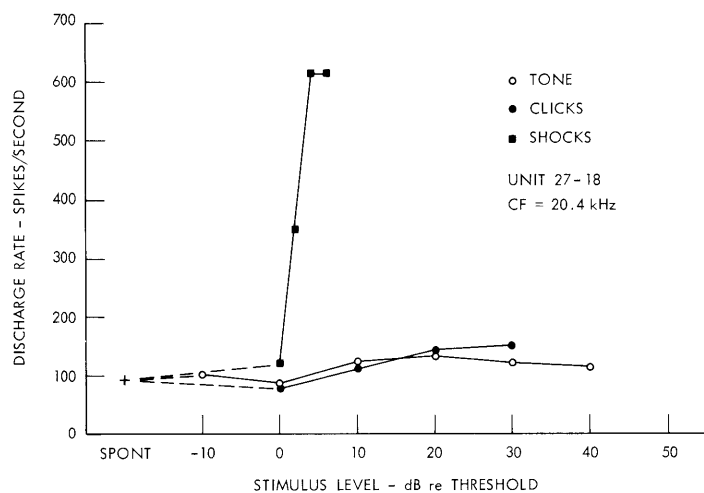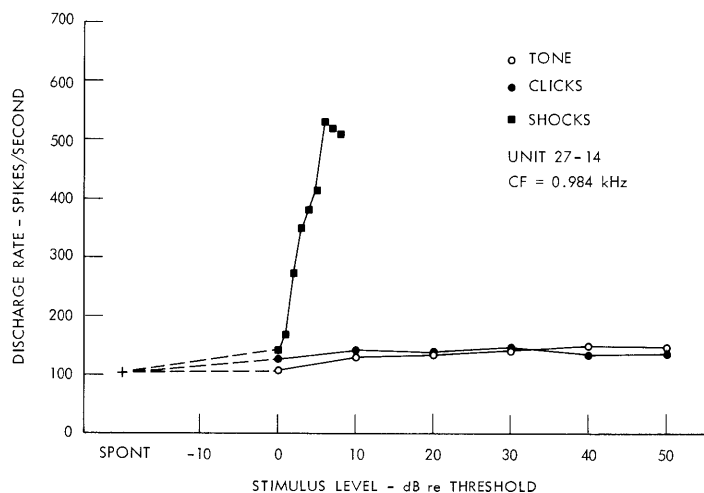
Fig. XXII-14. Discharge rate is plotted against stimulus level for units of high and low
CF. Repetition rate for clicks and shocks was 1000/sec. Stimulus level
is given in terms of dB relative to threshold. For unit 27-18, shock
threshold = 540 μA, tone threshold at CF = 51 dB SPL, click threshold,
approximately 80 dB SPL. For unit 27-14, shock threshold = 280 μA,
tone threshold at CF = 9 dB SPL, click threshold, approximately 35 dB
SPL. Both units exhibited relatively high rates of spontaneous discharge;
such units tend to exhibit the highest maximum rates with tonal stimuli
(see Kiang[1]).

With electric stimuli, however, rates of 500/sec or greater can be established and
have been maintained for several minutes. Apparently with acoustic stimulation func-
tional elements in the cochlea peripheral to the myelinated auditory-nerve fiber are
responsible for the limited discharge rates recorded in the nerve.

E. C. Moxon

References

1. N. Y. S. Kiang, Discharge Patterns of Single Fibers in the Cat's Auditory Nerve, M.I.T. Research Monograph No. 35 (The M.I.T. Press, Cambridge, Mass., 1965).

2. E. C. Moxon, "Electric Stimulation of the Cat's Cochlea: A Study of Discharge Rates in Single Auditory Nerve Fibers," S.M. Thesis, Department of Electrical Engineering, M.I.T., September 1967.

D. GUSTATORY POSTEXCITATORY DEPRESSION

In an earlier series of experiments involving repeated exposures of the tongue to a given gustatory stimulus, it was observed that the second application frequently elicited a reduced response, despite the fact that the tongue was rinsed with water for several seconds between applications. Thus it seemed as if the first exposure depressed the response to the second stimulus, while there were no observable changes in the base-line activity.

An investigation of the recovery of taste receptors from the effects of stimulation seemed to be of interest both from the standpoint of the biophysical mechanisms of taste stimulation and for developing proper methods of taste stimulation. To this end, the experiments described here were undertaken.

1. Methods

Thirteen cats were used. The animals were anaesthetized with sodium pentobarbitol (Diabutal). The initial dose was administered i.p. and sustained with i.v. doses. A lateral approach described by Cohen and his co-workers[1] was used in exposing the chorda tympani nerve. Recordings from the whole nerve were obtained with a silver electrode fixed in a micromanipulator. The nerve potentials were amplified by a Grass AC preamplifier, summated by an integrating circuit described by Diamant and his co-workers,[2] and recorded by a Sanborn DC polygraph, Model 67-1200.

The method of temperature control and application of solutions has been described previously by Hellekant.[3] The temperature of the water rinse and taste solutions was

Table XXII-1. Time constants (sec) of receptor recovery
functions for different taste solutions.

	$CaCl_2$		Choline Chloride	HAc	HCl	KCl	K_2SO_4	LiCl	Li_2SO_4	$MgCl_2$		NaCl			$NaNO_3$		NH_4Cl		Quinine Chloride	
	0.1	0.3	0.1	0.05	0.016	0.1	0.3	0.3	0.3	0.1	0.3	0.15	0.3	0.6	0.3	0.6	0.05	0.1	0.01	0.02
τ	19	23	9	4	6	5	5	13	20	23	25	13	10	6	25	16	3	4	4	3
n	7	1	4	4	2	3	3	3	1	6	1	4	6	4	4	4	6	2	2	1
τ_{min}	11	–	4	3	4	3	2	9	–	7	–	10	5	4	10	4	2	2	3	–
τ_{max}	32	–	20	5	8	6	7	18	–	40	–	15	12	8	45	22	6.5	6	5	–

kept at 27°C, except when the effects of higher temperatures were investigated. The stimuli were applied for approximately 3 sec, except when the effects of prolonged stimulation were studied. Solutions were made up in distilled water, which was also used to rinse the tongue. The water was applied constantly (5 ml/sec) between gustatory stimuli. Thirteen different chemicals were used as described in Table XXII-1. In the concentrations employed the various solutions elicited responses of approximately the same amplitude in the chorda tympani nerve.

2. Results

Figure XXII-15 shows a series of responses to 0.1-M $CaCl_2$ solution. The records were obtained from one animal during a single experiment. The paired stimuli with 9 different interstimulus intervals were presented in a random sequence. For clarity, the
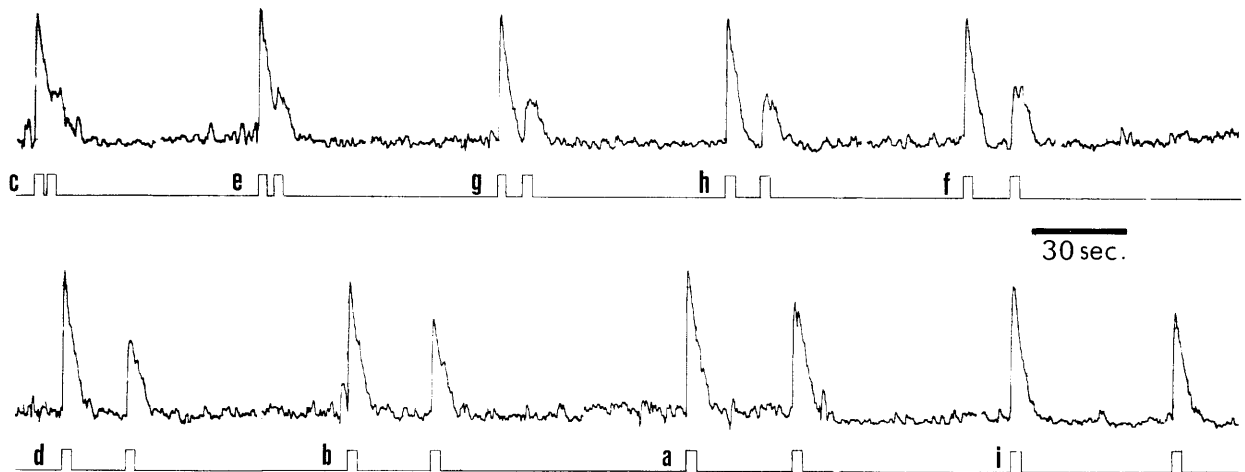

Fig. XXII-15. A series of summated responses to 0.1-M $CaCl_2$ solution in one cat. Actual order of presentation is indicated by the letters. Upward deflection of the signal shows the onset of stimulus; downward deflection, the onset of water. Flow rate: 5ml/sec.

records have been rearranged to show the effect of increasing interstimulus intervals. The actual order of presentation is indicated by letters a-i beside the first stimulus marker of each stimulus pair. The response to the right of the letter a in Fig. XXII-15 was recorded during the first exposure to $CaCl_2$ and serves as the control response of maximum amplitude under these recording conditions. It is evident from Fig. XXII-15 that the amplitude of the second response grew as the interstimulus interval was increased.

The amplitude of the response to the second stimulus of each pair in stimulus

sequences of this kind was then measured and plotted against the logarithm of the inter-stimulus interval, as was done in Figs. XXII-16, XXII-17 and XXII-18. The forms of these plots differed somewhat, dependent upon the chemical employed, its concentration, and the subject, as will be shown below. Imaginary curves through the points in these plots describe what will be called hereafter the receptor recovery functions (RRF).

a. Dependence of the RFF on Chemical

The duration of depression differed with the chemical used. The recovery after NH_4Cl, acids, and quinine hydrochloride was rapid. The recovery after potassium salts was somewhat slower. Recovery from choline chloride, sodium chloride, lithium salts, $CaCl_2$, $MgCl_2$, and $NaNO_3$ was progressively slower for these solutions in this order. This can be seen in Table XXII-1 where a time constant τ has been presented for each solution. This concept is used, despite the fact that the RRF plotted on a logarithmic time scale did not always display a straight line. τ was simply defined, therefore, as the time in seconds when a response that was 2/3 the amplitude of the maximum response was reached. The first row in Table XXII-1 shows the average time constant, ($\bar{\tau}$), for each solution; the second, the number of animals used in computing the τ value (n); the third, the shortest time constant observed for the solution (τ_{min}); and the fourth, the largest one (τ_{max}).

b. Dependence of the RRF on Concentration

Higher concentrations of a chemical usually gave a smaller τ value, which indicates a more rapid recovery. This is not always evident in Table XXII-1 because all animals were not tested with each solution. The variability related to individual differences was therefore not ruled out. On the basis of 12 observations, however, this relationship seemed to hold, except for $CaCl_2$ and $MgCl_2$. The RRF's for these solutions in several cases showed a continously increasing slope, an example of which can be seen in the left-hand graph of Fig. XXII-17. Higher concentrations seemed to deepen the upward concavity of the RRF's for these two chemicals. Consequently, their τ values increased when higher concentrations were used.

c. Dependence of the RRF on Subject

The slope of the RRF for a particular chemical differed from one animal to another. Some animals showed consistently shorter τ values than others. This is illustrated in the two right-hand graphs of Fig. XXII-16 which were obtained from different animals. The RRF for 0.6-M NaCl ought to be steeper than that for 0.3-M if concentration alone determined the slopes of the curves, but this is not the case. For a given animal, however, it appeared that higher concentrations yielded shorter recovery times. Furthermore, the sequence of the solutions (arranged according to their τ values for each
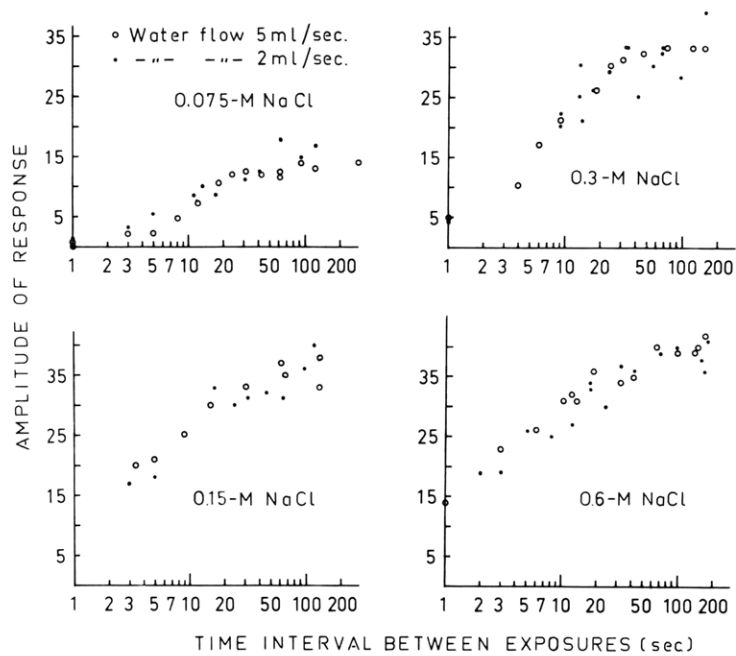
Fig. XXII-16. RRF's from 3 animals for a series of NaCl solutions with different flow rate for the intervening water rinse: 5 ml/sec and 2 ml/sec. The two bottom diagrams are from the same cat. The amplitude of each response in the series has been plotted against the logarithm of the time interval since the preceding stimulus.
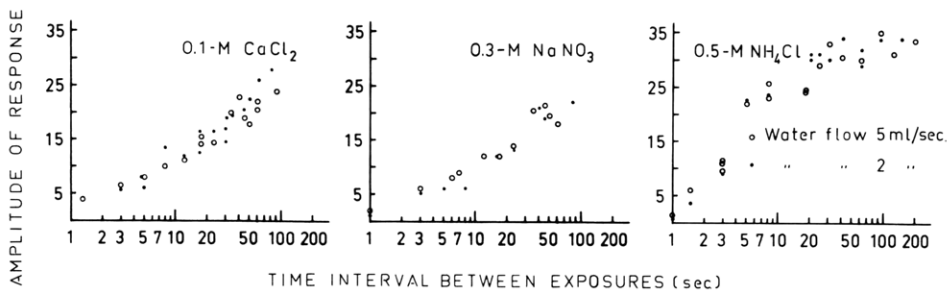

Fig. XXII-17. RRF's for three different chemicals and 2 flow rates of water rinse as in Fig. XXII-16. Recordings were obtained from the same animal.
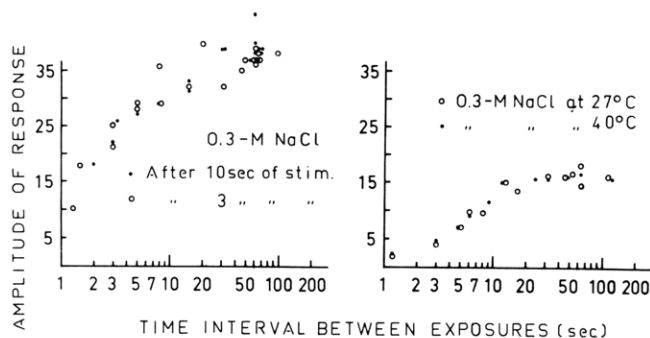

Fig. XXII-18. Left-hand graph shows RRF's for 2 series of 0.3-M NaCl solutions with 2 stimulus durations. Right-hand graph shows similar series with stimuli of different temperatures.

animal) was the same in almost all animals.

The τ value also varied to some extent when the same series of stimuli was repeated for individual subjects. The average variation was 17%, S.E. 3%.

d. Dependence of the RRF on the Intervening Water Rinse

It was possible that the results described above were due to an incomplete removal of the taste solutions from the tongue. Thus the diminished response might have resulted from a concentration difference between the remnants of the first stimulus and the second one. An attempt was made to test this possibility by diminishing the flow rate of the intervening water rinse. This should cause a slower recovery if the RRF's were very dependent upon this factor, since the sapid solution would be removed more slowly. Seventeen test series were run. Examples of the results are shown in the 7 graphs of Figs. XXII-16 and XXII-17. Responses from two series of stimuli, represented by dots and circles, are plotted in each graph. Interstimulus intervals ranged from 1 sec to 200 sec. The flow rate of the water rinse was changed from 5 ml/sec to 2 ml/sec and then back to 5 ml/sec. There is no consistent difference between the points for the two flow-rate conditions for different concentrations of NaCl (Fig. XXII-16) and three other chemicals (Fig. XXII-17). Sodium chloride was chosen because it is the most generally used salt stimulus in gustatory experiments, and has long been regarded as representing one of the basic taste qualities. $CaCl_2$ was chosen because of its rather different RRF described above. $NaNO_3$ and NH_4Cl were selected because they showed the fastest and slowest recoveries, respectively, after previous stimulation.

e. Dependence of the RRF on Length of Exposure

It might be suspected that the duration of the stimulus would affect the receptor in such a way that longer resting periods would be required after stimuli of longer durations. This question was examined briefly in the responses of 2 animals to 2 chemicals, NaCl and LiCl. The left-hand graph of Fig. XXII-18 shows the results obtained with NaCl.

The data are plotted in the same way as those in Figs. XXII-16 and XXII-17. The interstimulus intervals were the same for the two series, but the stimulus durations differed. The plots indicate no difference in recovery after stimulating for 3 sec or 10 sec with 0.3-M NaCl. Similar results were obtained with LiCl in the other series.

f. Dependence of the RRF on Temperature

The effect on the RRF of changing the temperature of sodium chloride solutions was also studied in one animal. The data are summarized in the right-hand graph of Fig. XXII-18 for a series of 0.3-M NaCl. The temperature of the stimulus and water rinse was 40° during one series, and 27° during the other. The experiments were

repeated several times on this animal with the same result. The data show no effect on the recovery functions with the 13°C change of temperature.

3. Discussion

It is possible to present a tentative explanation for the observations described above. This explanation is based on Beidler's adsorption theory.[4,5] Beidler assumed that the reversible binding between stimulus and receptor sites on the microvilli provides the basis for gustatory sensation. There are many kinds of receptor sites and each is adapted to one kind of stimulus. Competition among stimuli occurs if they are presented together and resemble each other from the receptor's point of view. Experimental observations have shown a relatively fast reaction between most stimuli and their receptors, less than 1 sec if the duration of the transient response is used as a measure (see Zotterman[6]). It is conceivable that the rate of disengagement between receptor sites and stimulus is slower than the rate of engagement; that is, the affinity between sites and stimulus is greater than that beween water and the stimulus. Moreover, the affinities between stimuli and their sites differ (see Beidler[5]). It is therefore rather obvious that the rates of disengagement between stimuli and their sites may differ. This would be observed as variations in the RRF's for different chemicals.

It has also been observed that the duration of the postexcitatory depression is lengthened following prolonged stimulation (cf. Granit[7]). This is in contrast to the observations in the present study which indicated that an increase in stimulus duration from 3 sec to 10 sec had no effect on the postexcitatory depression (RRF). Beidler's general taste equation[4] was derived from the concept of an equilibrium that is achieved between the number of unoccupied and occupied receptor sites. This concept can be used here if it is assumed that the reaction between the stimulus and its sites reaches equilibrium within 3 sec. Consequently, extended stimulation should not increase the number of occupied sites and would have no effect on the recovery after stimulation. The time course of the electrical responses during stimulation with the substances used in this study indicates also that the adsorption between the stimulus and its receptor sites reaches an equilibrium within 3 sec, as the responses by then reach a steady or slowly declining state. Three seconds have also been shown to be long enough for gustatory sensation (see Marcström[8]).

This study indicates an effect of different stimulus concentrations on the RRF, since it was observed that a higher concentration usually gave a smaller τ value. This may seem paradoxical because it is implicit in the adsorption theory that during stimulation a stimulus of stronger concentration will occupy a larger number of the available receptor sites than a weaker stimulus. Fewer receptor sites will then be available after stimulation with a stronger stimulus. This should cause a larger depression of the response following the stronger stimulation, provided the same concentration is used for the second stimulus in both cases, which is also the case, as will be shown in

a later study. But in the present series the strength of the second stimulus was not kept constant, but increased to the same level as the first; that is, the same stimulus was repeated. This complicates the situation, as the greater depression caused by the stronger first stimulus will be counteracted by the increase in strength of the second stimulus. In this study the τ values, in general, became smaller when both concentrations were raised; this may indicate that the increase of stimulus strength during the first two-thirds of the recovery outweighed the increased depression.

The influence of temperature on the adsorption between a salt stimulus and its receptor sites is small when judged by the neural response (cf. Beidler[9]), especially in the temperature range 30-40°C (cf. Yamashita, Yamada and Sato[10]). The effect of temperature changes on the rate of disengagement between the stimulus and its receptor sites is also probably small and, therefore, not noticeable within this temperature range. It may therefore explain the absence of an effect on the RRF when the temperature was raised from 27°C to 40°C.

Each taste bud is composed of several gustatory cells that, by means of microvilli, project into the taste pore. The taste pore is open to the solution around the papilla. Ultramicroscopical observations by Trujillo-Cenóz,[11] DeLorenzo,[12] and Nemetscheck-Gansler and Ferner[13] show that microvilli do not project outside the taste pore. The last authors also state that there is a space between the mouth of the taste pore and the microvilli. Beidler suggested[5] that the taste stimulus within the taste pore is transported to and from the membrane of the microvilli by diffusion. He regarded the importance of diffusion as small during stimulation. But, because of the time course of diffusion, it will take longer to achieve a low-level or threshold concentration when the stimulus is removed than when it is applied. A comparison between the order of diffusion velocities of the substances used and their RRF's is therefore of value when evaluating this factor. The order of diffusion velocities of the inorganic salts (see Renqvist[14]) agreed with the order of recovery after stimulation in the present experiments, except for $NaNO_3$ which showed the slowest recovery curve, although its calculated diffusion velocity is relatively fast. Choline chloride and quinine chloride, however, have substantially lower diffusion velocities than the other substances, but recoveries after these stimuli were among the fastest. It is therefore not very likely that diffusion time is the main factor in the recoveries observed.

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