J. Physiol. (1971), **212**, *pp.* 593–609
With 6 text-figures
Printed in Great Britain

PROPERTIES OF 'TWO-TONE INHIBITION' IN PRIMARY AUDITORY NEURONES

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(*Received 22 April 1970*)

SUMMARY

1. Properties of two-tone inhibition in primary auditory neurones of cats were studied with phase-locked sound stimuli. One sound was a continuous tone at the best frequency of a given neurone, and the other, a tone burst which was changed in amplitude, frequency, and phase relative to the continuous tone.

2. The tone burst which caused two-tone inhibition had either an excitatory or no effect when it was delivered alone. Inhibitory areas commonly appeared on both sides of the excitatory area when the best frequency was higher than a few kc/s.

3. Two-tone inhibition began and ceased within a few milliseconds of the onset and termination of the excitation caused by a tone burst. The degree of inhibition was greatest at the beginning of the tone burst and reached a plateau within 500 msec. The discharge rate during inhibition could be lower than the rate for either tone alone or for spontaneous activity. At the termination of inhibition, prominent rebound in the discharge rate was found.

4. With an increase in amplitude of a tone burst, for either a fixed or equally increased continuous tone, the discharge rate during inhibition decreased to a minimum and then began to increase. That is, the degree of inhibition was non-monotonically related to the sound level.

5. Compound period histograms of discharges during inhibition showed that single neurones usually carried information about the combined wave form of the two tones. The information about each tone was, however, modified by the inhibitory phenomenon in both amplitude and phase from that indicated by the compound period histograms for the individual tones.

6. Possible mechanisms and functional significance of two-tone inhibition are discussed.

INTRODUCTION

A *period histogram* represents the change in the probability of discharge as a function of time for an interval of one or a few cycles of a periodic stimulus. It has been shown for mammalian primary auditory neurones that the shape of the period histogram resembles the input wave form for sinusoidal signals below about 4 kc/s (Hind, Anderson, Brugge & Rose, 1967; Brugge, Anderson, Hind & Rose, 1969; Goblick & Pfeiffer, 1969). If two tones are present in the stimulus, the shape of the period histogram approximates the wave form obtained from the addition of the component sinusoids with some modification in amplitude and phase. This observation suggests that the shape of the period histogram approximately represents the motion of the basilar membrane. But under certain conditions the neural response may depend upon factors in addition to the membrane motion. For example, in monkeys (Katsuki, Suga & Kanno, 1962; Nomoto, Suga & Katsuki, 1964), cats (Rupert, Moushegian & Galambos, 1963; Sachs & Kiang, 1968; Sachs, 1969) and bats (Frishkopf, 1964), it has been found that the discharge rate of a primary neurone for a continuous pure tone at its best (or characteristic) frequency is often reduced by the simultaneous delivery of a tone burst. This phenomenon is called either 'peripheral', 'direct' or 'two-tone' inhibition. The inhibitory areas in which the response to the sound at the best frequency is reduced by another sound appear on one and often on both sides of the excitatory (or response) area of the neurone.

Mechanisms of this inhibition are not presently clear and could be related to either (*a*) mechanical or transducer events, (*b*) neural events, or (*c*) both. One should understand the properties of inhibition and its mechanism in order to help define its origin and to determine its role in information processing in the cochlea. To approach these objectives, studies were made of (1) the discharge rate and discharge pattern during periods of inhibition elicited by two phase-locked sinusoidal stimuli, and (2) the latencies of onset and termination of inhibition.

METHODS

Twenty adult cats were anaesthetized with intraperitoneal injections of Dial with urethane (0·75 mg/kg). They were placed in a sound-quieted chamber and monaurally stimulated using a Brüel and Kjaer 4133 condenser microphone with distortion compensating driver amplifier (Molnar, Loeffel & Pfeiffer, 1968). The sound pressure level (db SPL ref. 0·0002 dyne/cm^2) was measured in proximity to the eardrum with a calibrated probe microphone. The cochlear nerve was exposed by retracting the cerebellum medially. Micro-electrodes filled with 3 M-KCl were inserted into the nerve in the internal auditory meatus. Phase-locked sound stimuli were controlled and the

responses to them processed with a Laboratory Instrument Computer (LINC) (Clark & Molnar, 1964).

When the activity of a single neurone was isolated, its best (or characteristic) frequency and threshold at that frequency (see Fig. 2) were determined by using either 100 msec tone bursts with a rise–decay time of 2·5 msec and a repetition rate

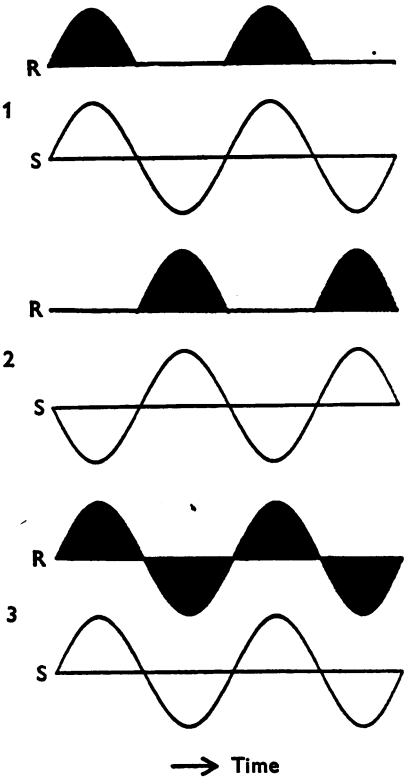

Fig. 1. The compound period histogram. The number of discharges which occurred at any time after the beginning of a cycle of the stimulus (1, S) was counted over a few following cycles. This procedure was repeated for many successive groups of cycles until the shape of the period histogram (1, R) became clear. The polarity of the stimulus was reversed (2, S) and another period histogram obtained (2, R). The two period histograms were added after inverting the one for which the polarity of the stimulus had been reversed (3, R). The shape of the compound period histogram was then found to represent the stimulus wave form (3, S).

of 5/sec, or a frequency-modulated signal, in which frequency was swept 1·4 decades upward then downward three times during a 60 sec period. Subsequently, both a continuous tone at the best frequency (at a level 10–20 db above the threshold) and either a tone burst or frequency-modulated signal were delivered simultaneously. The continuous tone and tone burst were phase-locked; the continuous tone and frequency-modulated signals, of course, were not. Post-stimulus–time histograms of

responses to either the continuous tone, frequency-modulated signal, tone burst, continuous tone plus frequency-modulated signal, or continuous tone plus tone burst were calculated, displayed, and stored by the LINC. The histograms were synchronized to the beginning of the upward sweep of the frequency-modulated signal. Post-stimulus–time histograms taken for studies with the frequency-modulated stimuli were utilized to measure the excitatory and inhibitory areas (Sachs & Kiang, 1968). The threshold of excitation was *arbitrarily defined* as the lowest signal level at which the discharge rate was about 20 % above the background rate; and the threshold of inhibition, as that at which the second tone decreased the discharge rate for the first tone by about 20 %.

In the continuous tone plus tone burst combination, post-stimulus–time histograms with a 0·8 msec bin width were used to measure the extent of inhibition as a function of signal amplitude and relative phase between the two tones. To obtain the difference in latency between inhibition and excitation at both the beginning and end of the tone burst a post-stimulus–time histogram with a 0·048 msec bin width was employed. The resolution of the latency difference measurement at the termination of the tone burst was comparable to the bin width, since the duration of the tone burst was stable to within 0·1 msec/hr. Adaptation was examined by taking post-stimulus–time histograms with a 16 msec bin width for 2 sec tone burst stimuli. To study discharge patterns, period histograms were made for normal and reversed polarity signals (Fig. 1, records 1 and 2) and put together in a *compound period histogram* as shown in Fig. 1, record 3 (Goblick & Pfeiffer, 1969).

RESULTS

Discharge rate during inhibition

When a tone burst was delivered with a continuous tone at the best frequency, the response was often less than that to either the continuous tone or tone burst alone. By changing the frequency and amplitude of the tone burst, inhibitory areas were measured in which the response was less than that to the continuous tone alone. For neurones with best frequencies higher than a few kc/s, inhibitory areas were usually seen on both sides of the best frequency (Fig. 2). These observations are consistent with those of Sachs & Kiang (1968). However, two-tone inhibition was often not clear on either side in neurones with best frequencies lower than a few kc/s. When observed, the inhibitory areas commonly appeared on both sides of the excitatory area and slightly overlapped it. Thus, the tone burst had either an excitatory or no effect on a given neurone when it was delivered alone. Hereafter, a tone burst that produced inhibition of the response to the continuous tone and which had an excitatory effect is called an excitatory tone burst, and that which had no excitatory effect, a non-excitatory tone burst.

The average discharge rate during the delivery of a non-excitatory tone burst alone was not less than the spontaneous discharge rate, although it has been reported that this occurs in a small percentage of neurones (Katsuki *et al.* 1962; Rupert *et al.* 1963). The rate of discharges during two-

tone inhibition was, however, sometimes lower than the spontaneous rate (Fig. 3 *Ab* 4 and *Bb* 3). When the frequency of an excitatory tone burst was fixed and its amplitude increased, the number of spike discharges in the response increased monotonically over a certain dynamic range (Fig. 3 *Aa* and *Ba*). However, the number of spikes during two-tone inhibition did not decrease monotonically in the same range (Fig. 3 *Ab*). As the amplitude of the excitatory tone burst was increased, the response to the

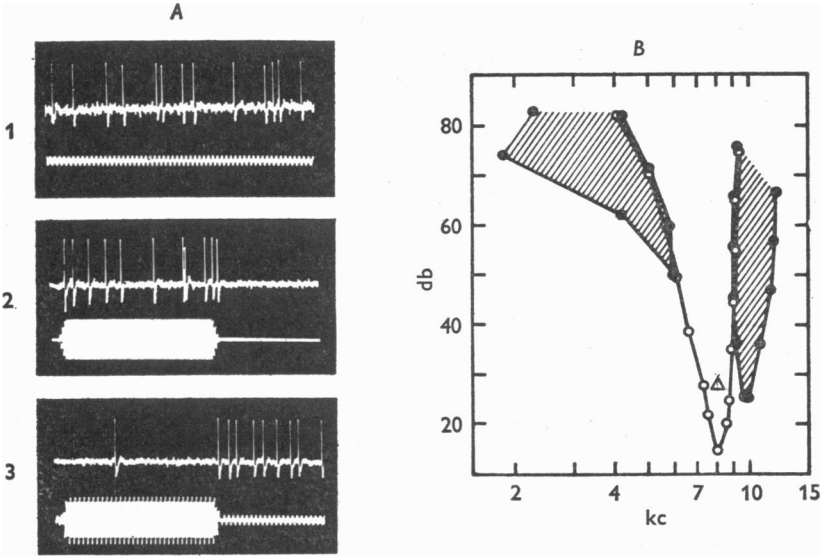

Fig 2. An example of two-tone inhibition (*A*) and excitatory and inhibitory areas (*B*). *A*: a single primary auditory neurone responds to a 40 db continuous tone at the best frequency of 360 c/s (*A* 1), and also to a 60 db tone burst of 720 c/s (*A* 2). When these two tones are simultaneously delivered, inhibition is demonstrated (*A* 3). The duration and rise–decay time of the tone burst are 100 and 2·5 msec, respectively. *B*: the ordinate represents sound pressure level (db referred to 0·0002 dyne/cm²) and the abscissa, stimulus frequency in kc/s. The area above the tuning curve (open circles) is the excitatory one in which the discharge rate is more than 20 % above the spontaneous rate. The hatched areas are inhibitory ones in which the response to a continuous tone at the best frequency (triangle) plus a second tone is more than 20 % below the response to the continuous tone alone.

burst became predominant (Fig. 3 *Ab* 1 and 2). For cases in which the continuous tone was strengthened while keeping the burst at a fixed amplitude, the response to the tone became predominant. When the difference in signal amplitude between the continuous tone and the excitatory tone burst was kept constant and both simultaneously decreased (Fig. 3 *Bb*), inhibition became clearer and almost complete (Fig. 3 *Bb* 3). Further attenuation made the inhibition less prominent (Fig. 3 *Bb* 4 and 5).

Systematic changes in the phase relation between the continuous tone and tone burst did not change the degree of inhibition in most neurones, although the temporal structure of the discharges was strongly phase dependent as described below. In a few neurones, the effect of a change in phase on the degree of inhibition was similar to that reported for frog primary auditory neurones (Liff, 1970).

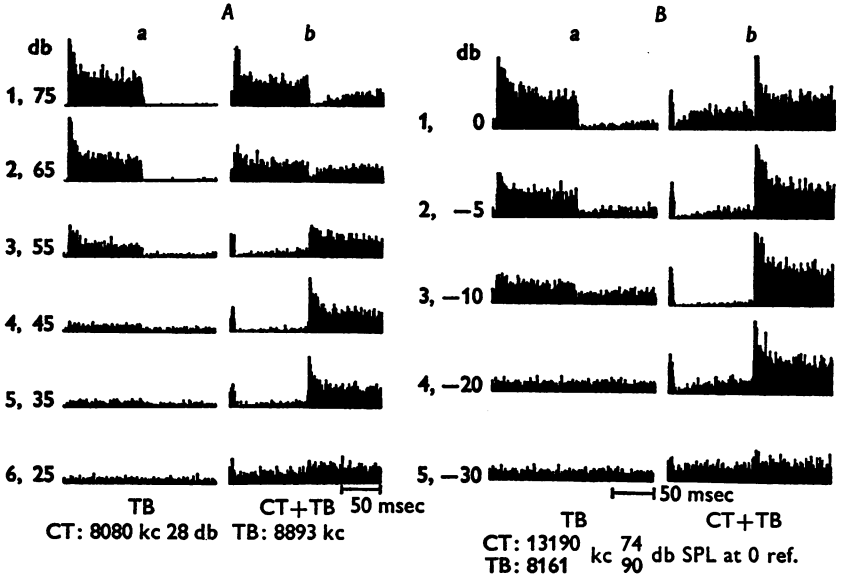

Fig. 3. Post-stimulus–time histograms for responses of two single neurones (*A* and *B*). *Aa* and *Ba* show responses to 100 msec tone bursts presented 128 times at a rate of 5/sec. *Ab* and *Bb* show responses to a combination of a continuous tone at the best frequency and the tone burst. In *Ab*, the amplitude of the continuous tone was fixed and that of the tone burst changed. In *Bb*, both the continuous tone and tone burst were equally changed in amplitude. In each case, as the amplitude was decreased, inhibition became almost complete (*Ab*4 and *Bb*3) and then incomplete. The frequencies and amplitudes of the stimuli used are shown in kc/s and db SPL.

Time course of inhibition

The temporal relations of discharges at both the onset and termination of two-tone inhibition were examined to attempt to distinguish between the roles of mechanical, local neural, and higher neural mechanisms (Figs. 4 and 5). Inhibition occurred after the onset of the tone burst with a latency shorter than that of inhibition evoked by efferent fibre activity as reported by Fex (1962). Fig. 4 (On) shows post-stimulus–time histograms of the response at the onset of excitatory tone burst for the combination of continuous tone plus excitatory tone burst and for the latter alone.

The beginning of inhibition and excitation are marked according to the definitions given in Fig. 2. Inhibition may occur either before or after the excitatory tone burst evokes excitation. For example, in Fig. 4*a*, inhibition

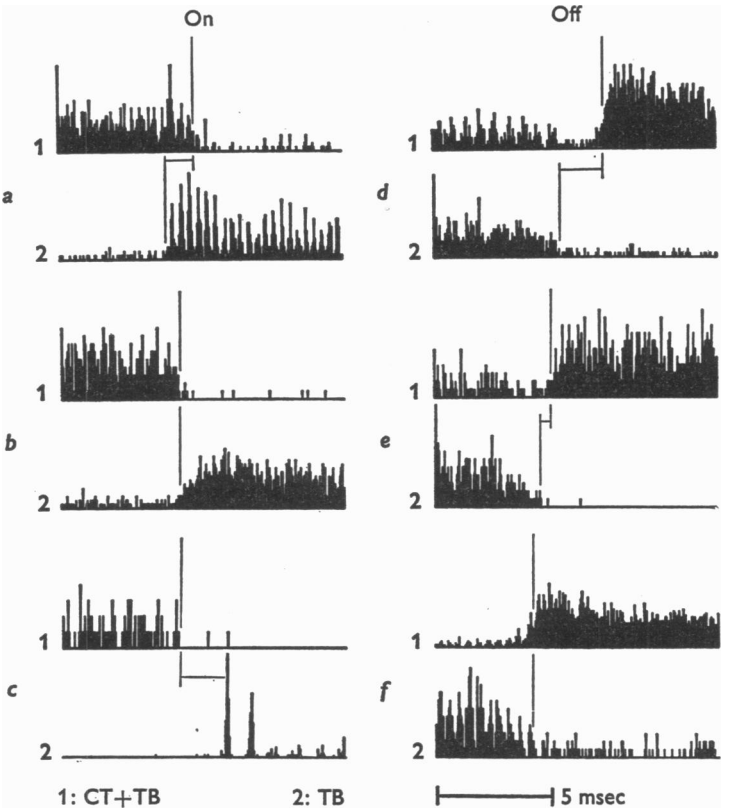

Fig. 4. Difference in latency between inhibition and excitation. Post-stimulus–time histograms are shown with the response to a combination of continuous tone at the best frequency plus tone burst in 1 (vertical line indicates beginning or end of inhibition) and the response to the tone burst alone in 2 (vertical line indicates beginning or end of excitation) for six single neurones (*a–f*). The stimulus was repeated 1024 times for each histogram. The rise–decay time of the tone burst was 2·5 msec. The bin width is 0·048 msec. The frequencies and levels of the stimuli in kc/s and db SPL are: (*a*) Continuous tone: 7·703, 50; tone burst: 2·773, 90. Inhibition occurred 1·4 msec after excitation. (*b*) Continuous tone: 7·703, 30; tone burst: 4·806, 77. Inhibition occurred at the same time as excitation. (*c*) Continuous tone: 17·785, 72; tone burst: 980, 97. Inhibition occurred 2·4 msec before excitation. (*d*) Continuous tone: 9·613, 57; tone burst: 11·274, 91. Inhibition terminated 2·1 msec after excitation. (*e*) Continuous tone: 7·409, 44; tone burst: 5·748, 72. Inhibition terminated 0·6 msec after excitation. (*f*) Continuous tone: 6·998, 43; tone burst: 8·814, 81. Inhibition terminated at the same time as excitation.

occurs 1·4 msec after excitation. In Fig. 4*c*, it occurs 2·4 msec before excitation. As the amplitude of the tone burst increases, it can be seen from Fig. 2 that the combination continuous tone plus tone burst enters the inhibitory region before the excitatory tone burst initiates excitation. Thus the 2·5 msec rise time would be expected to introduce a delay in the excitation relative to inhibition. Fig. 5*A* is a plot of the latency difference

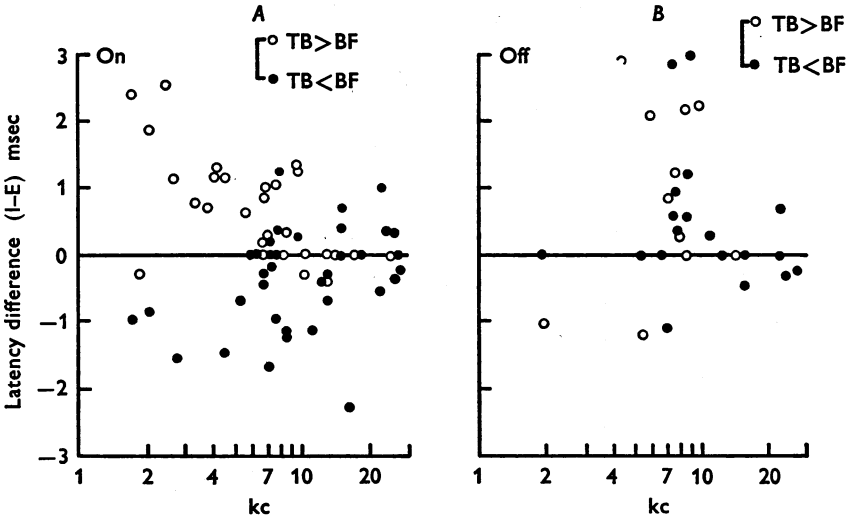

Fig. 5. Time difference between inhibition evoked by a continuous tone at the best frequency plus tone burst and the excitation evoked by the tone burst alone at both its onset and termination. Open circles indicate that the frequency of the tone burst was higher than the best frequency; filled circles, the frequency of the tone burst was lower. The rise–decay time of the tone burst was 2·5 msec. At the onset of the tone burst (*A*), the range of time differences between inhibition and excitation in fifty-eight neurones is about ± 2 msec. At the termination of the tone burst (*B*), the range of values in twenty-seven neurones is from about − 1 to + 3 msec.

between inhibition and excitation at the onset of a excitatory tone burst in fifty-eight neurones. Most latency differences are between + 2 and − 2 msec. There does not seem to be a bias favouring the negative latency differences expected as a consequence of the 2·5 msec rise time of the excitatory tone burst. The apparent tendency for the values to be positive for excitatory tone-burst frequencies higher than the best frequency and negative for lower excitatory tone-burst frequencies was not verified in measurements on the same neurones using both faster and slower rise times (1·0 and 5·0 msec) for the excitatory tone burst. However, the range of the latency differences remained unchanged.

After the onset of inhibition the discharge rate gradually increased and reached a plateau within 500 msec. When the excitatory tone burst was turned off, the inhibition terminated as shown in Fig. 4 (Off). The excitation for the burst usually ended before the response increased in the continuous tone plus excitatory tone-burst histogram. In Fig. 4*d*, inhibition terminates 2·1 msec after excitation. However, there was a simultaneous decrease in the discharge rate in this histogram as the response to the excitatory tone burst alone ceased. In Fig. 4*f*, inhibition and excitation end almost at the same time. As the amplitude of the tone burst decays, the latter leaves the excitatory area before the inhibitory area (see Fig. 2). Therefore the 2·5 msec excitatory tone-burst decay should cause the latency difference to be longer than would be anticipated for an abrupt decay. Fig. 5*B* shows the latency differences in twenty-seven neurones. The range is approximately -1 to $+3$ msec. The 4 msec spread is the same as that observed at the onset of the excitatory tone burst. The preponderance of positive values in the latency differences may be considered a result of the decay time of the burst.

The latency measurement was complicated by the requirement that the tone burst rise–decay had to be smoothed to avoid a transient response that obscured both the onset and termination of inhibition. The rise–decay times that were used were on the order of the observed latency differences. Interpretation of the results was difficult because the variation in the latency difference with a change in the rise–decay time was not clearly systematic. Thus, the simultaneous occurrence of inhibition and excitation may be compatible with these results.

Following inhibition, the discharge rate quickly rebounded beyond the steady-state discharge rate for the continuous tone with a time course similar to that of the response to a tone burst. The steady-state discharge rate was reached within 500 msec.

Discharge patterns during inhibition

When a single sinusoid below about 4 kc/s was delivered, discharges were correlated to the phase of the stimulus. This 'phase locking' was clearly shown during inhibition using the technique of the compound period histogram. The amplitude and frequency of both the continuous tone and tone burst were adjusted for incomplete inhibition, so that the relation in time between the occurrence of discharges and the wave form of the stimulus could be seen. Parts *A* and *B* of Fig. 6 show patterns obtained for non-excitatory and for excitatory tone-burst stimuli, respectively. Patterns from six neurones are presented. Post-stimulus–time histograms were first obtained for each neurone to determine the degree of two-tone inhibition as shown in Fig. 6 *Aa* and *Ba*. Then compound

period histograms were made by sampling the discharges during periods designated by 'p' in the post-stimulus–time histograms for continuous tone, tone burst and these combined stimulations. Each wave form of the continuous tone was approximated by the compound period histogram (Fig. 6*A*, *b*1, *c*1, *d*1, and 6*B*, *b*1, *c*1, *d*1). The wave form of the tone burst was also reproduced by the histogram for the excitatory tone bursts (Fig. 6*B*, *b*2, *c*2, *d*2). The way in which the non-excitatory tone-burst

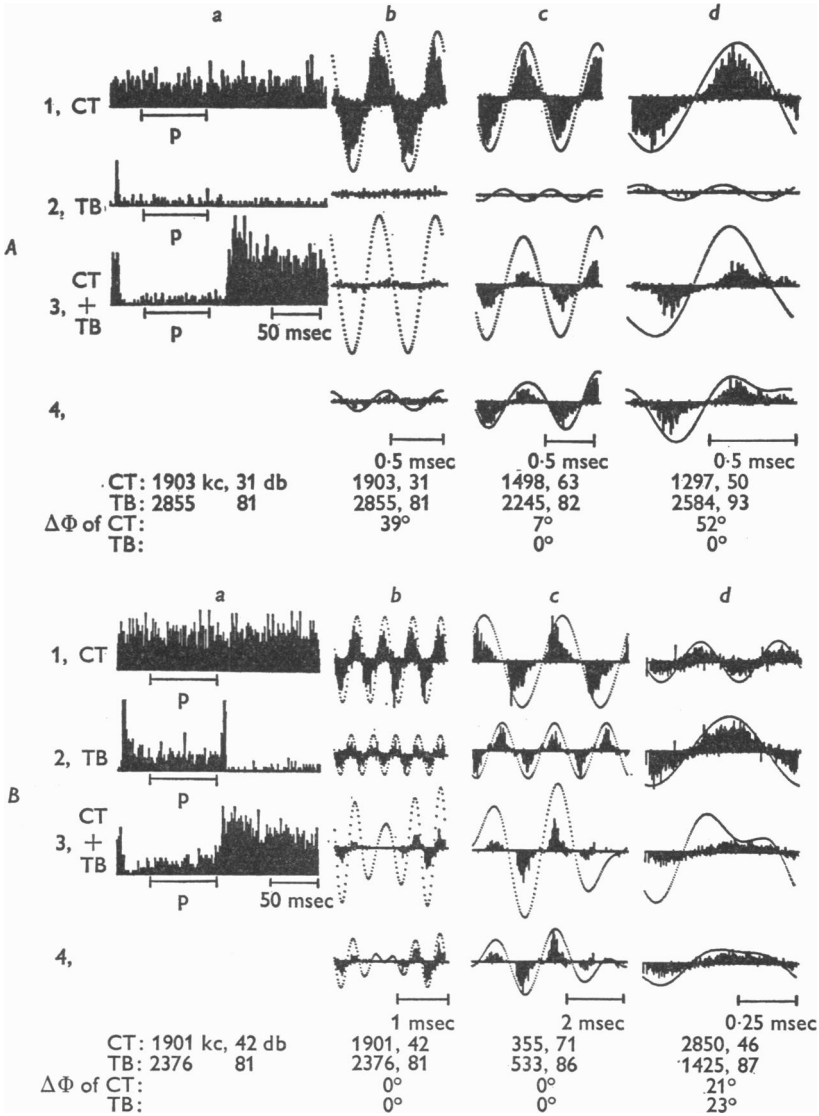

Fig. 6. For legend see opposite page.

wave forms (Fig. 6*A*, *c*2, *d*2) were determined is described below. The two stimuli were simultaneously delivered without changing any parameters, including phase. The compound period histogram during inhibition was quite different from the wave form of the two-tone stimulus obtained by adding the continuous tone and tone-burst wave forms (Fig. 6*A*, *b*3, *c*3, *d*3, and 6*B*, *b*3, *c*3, *d*3). These two wave forms were adjusted for the best fit to the compound period histogram (Fig. 6*A*, *b*4, *c*4, *d*4, and 6*B*, *b*4, *c*4, *d*4).

In Fig. 6*Ab* discharges during the non-excitatory tone bursts provide no information about the stimulus wave form (*Ab*2). The compound period histogram for the two-tone stimulus showed a remarkable change in amplitude and phase from that for the continuous tone alone (*Ab*3). In order to fit the histogram, this in *b*1 was reduced by 17 db and shifted by 39° (*Ab*4). The fit was not improved by adding the non-excitatory tone-burst wave form at any level or phase. In Fig. 6*Ac*, an example is shown in which the shape of the compound period histogram during inhibition reflects the presence of the non-excitatory tone-burst frequency. The discharges associated with no effect again provide no information about the stimulus wave form (*Ac*2). The non-excitatory tone-burst wave form in *Ac*2, however, was required to fit the histogram during inhibition (*Ac*4). The tone-burst wave form was reduced by 7db and shifted by 7° from that for the continuous tone alone. In Fig. 6*Ad* the non-excitatory tone burst is subthreshold according to our definition, but periodically reduced

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Fig. 6. Discharge pattern during inhibition for non-excitatory tone-burst stimuli in *A* and excitatory tone-burst stimuli in *B*. *a*. Post-stimulus–time histograms for continuous tone, tone burst, and both combined are shown in 1, 2 and 3 respectively. These were used to determine the degree of inhibition. *b*. Compound period histograms in 1, 2, and 3 are composed of discharges taken over the region of the histogram marked p. *c* and *d*. Compound period histograms measured in two other neurones. In row 1, the compound period histograms show the response to the continuous tone with the stimulus wave form superimposed. The wave form of the continuous tone was adjusted in amplitude and phase until it matched the shape of the histogram. The same procedure was followed for the tone burst in row 2 whenever a wave form could be determined. In row 3, the compound period histogram for the continuous tone plus tone burst is shown with the wave form which results from a summation of the wave forms in rows 1 and 2. In row 4, the same histogram is shown with the amplitudes and phases of the continuous tone and tone-burst wave forms adjusted for best fit. The frequencies and amplitudes of the stimuli are shown in kc/s and db SPL. $\Delta\Phi$ represents a phase shift in degrees. The frequency ratio of continuous tone to tone burst and the number of fundamental periods over which discharges were sampled was 3:2 and 40,960, respectively, in *Ab*; 3:2 and 57,344 in *Ac*; 2:1 and 49,052 in *Ad*; 5:4 and 8,192 in *Bb*; 3:2 and 3072 in *Bc*; 1:2 and 28,672 in *Bd* (see text).

background activity so that its phase could be established (*Ad* 2). The continuous tone and non-excitatory tone burst were simultaneously delivered with this phase relation. The compound period histogram for the two-tone stimulus is quite different in amplitude and phase from that for the continuous tone alone (*Ad* 3). By adjusting both the level and phase of the continuous tone and tone-burst wave forms in *Ad* 1 and *Ad* 2, it was possible to fit the wave form of the two-tone stimulus to the compound period histogram (*Ad* 4). To obtain the fit the continuous tone had to be attenuated by 6 db and shifted by 52°, the non-excitatory tone burst had to be increased by 6 db. Although not shown, a shift in the phase during inhibition from that for the non-excitatory tone burst alone was observed among some of the cases for which the phase could be fixed as in Fig. 6*Ad*.

In Fig. 6*Bb* wave forms of both the continuous tone and excitatory tone burst are represented by the histograms (*Bb* 1 and *Bb* 2). The post-stimulus–time histogram for the two-tone stimulus clearly shows inhibition (*Ba* 3). The addition of the wave forms in *Bb* 1 and *Bb* 2 clearly differs from the histogram during inhibition (*Bb* 3). When the continuous tone and tone burst were attenuated by 10 and 3 db, respectively, the combined wave form matched the compound period histogram (*Bb* 4). It was not necessary to adjust the phase relation of either wave form. In Fig. 6*Bc* the wave forms which fit the continuous tone and excitatory tone-burst histograms (*Bc* 1 and *Bc* 2) had to be reduced by 8 and 4 db, respectively, in order to fit the histogram for the two-tone stimulus (*Bc* 4). No phase change was required. The example in Fig. 6*Bd* shows an excitatory tone-burst frequency lower than the continuous tone frequency. The continuous tone and tone-burst wave forms matched the compound period histograms in *Bd* 1 and *Bd* 2. The sum of the wave forms is shown in *Bd* 3. In order to fit the histogram during inhibition the continuous tone and excitatory tone-burst wave forms had to be attenuated by 11 and 6 db, respectively (*Bd* 4). In *Bd* 4 the continuous tone phase was shifted by 21° from that for the continuous tone alone, and the tone-burst phase was shifted by 23° from that for the excitatory tone burst alone.

The match obtained in the fitting procedure was unique. A variation of 2 db in the amplitude or a phase change equivalent to one or two bin widths for either component appreciably reduced the quality of the fit. The phase resolution ranged from 2° (Fig. 6*Ad*) to 10° (Fig. 6*Bb*).

Thus inhibition was not due to the simple attenuation of the response to the continuous tone or of the sum of the two signals. Since the shape of a compound period histogram could be approximated by the wave form obtained by combining the two stimulus wave forms, it is apparent that single neurones carried information about both tones during inhibition.

The information, however, was greatly modified in amplitude and phase by the inhibitory phenomenon compared to that observed in the histogram for each of the tones alone.

DISCUSSION

Any mechanism proposed to explain two-tone inhibition should account for the following observations:

(1) *The onset and termination.* Inhibition begins and ends within a few milliseconds of the beginning and end of the second tone (Figs. 4 and 5).

(2) *Adaptation and rebound.* The degree of inhibition is greatest at the beginning of the second tone and reaches a plateau within 500 msec. At the termination of inhibition, prominent rebound of discharges is found (Fig. 3).

(3) *Nonmonotonic inhibition.* With an increase in the amplitude of a tone burst for either a fixed or equally increased continuous tone amplitude, the discharge rate during inhibition decreases to a minimum and then begins to increase (Fig. 3).

(4) *Inhibition by a non-excitatory tone burst.* Inhibition may be produced by a second tone that causes no effect on the background activity when presented alone (Figs. 2 and 6*A*).

(5) *Inhibition by an excitatory tone burst.* The discharge rate during two-tone inhibition can be lower than the discharge rate produced by either tone alone (Figs. 3 and 6*B*).

(6) *Latency difference.* The latency of inhibition may differ from that of excitation to an excitatory tone burst. It may be either longer or shorter, but the difference is typically less than 2 msec (Figs. 4 and 5).

(7) *No inhibition of spontaneous discharges.* Although it may occur in a small percentage of neurones, inhibition of spontaneous activity by a single tone was not observed.

(8) *Discharge pattern.* The shape of the compound period histogram during inhibition can be approximated by a wave form containing the two frequency components present in the stimulus (Fig. 6).

(9) *Phase shift.* The phase of either the continuous tone or tone burst component of the wave form which fits the compound period histogram during inhibition may differ from that of the wave form which fits the compound period histogram of that tone alone (Fig. 6).

In the following, possible mechanisms and the functional significance of two-tone inhibition are considered:

Simple mechanical disturbance

'Inhibition by an excitatory tone burst' might be explained simply if the motion of the basilar membrane generated by the continuous tone is

amplitude modulated by the presence of the excitatory tone burst, so that the discharge rate becomes smaller in one phase, but larger in another, compared to that for the continuous tone alone. However, the compound period histograms during inhibition clearly show that this simple explanation does not hold. In addition, 'inhibition by a non-excitatory tone burst' is difficult to reconcile with a simple disturbance of the membrane motion produced by the continuous tone, since the non-excitatory tone burst presumably vibrates the membrane at the site of innervation by a given neurone in subthreshold amplitude. Finally, inhibition by a mechanical disturbance at a single site would be expected to begin and end at the same time as excitation by the tone burst. The observed 'latency differences' were not zero. Thus the mechanism of attenuation of responses by the simultaneous delivery of two tones may involve other aspects of the peripheral auditory system such as the non-linearity of system elements and the possibility of neural interaction.

Non-linearities of the peripheral system

Since inhibition is observed when two tones are added, a non-linearity in the input–output relation of an element between the tympanic membrane and the primary auditory neurones may result in the decrease of excitation to the neurones for an input beyond a certain sound pressure level. The response rate of neurones versus the input level for a single tone stimulus may reach a peak and then decrease (Kiang, 1965). This concave downward relation may span more than a 100 db range in some neurones. If two-tone inhibition is due solely to the increase in input level when both tones are present, inhibition would become more prominent as the amplitude of the continuous tone or tone burst or both are increased. The inhibition, however, usually became obscure with an increase in either or both levels.

When two frequency components are present in the stimulus, the effect of a non-linearity will be different from that for a single tone if the stimulus is coupled to an element, such as the cochlear partition, which filters the signal. The energy in a continuous tone or tone burst in the two-tone stimulus, after distortion and filtering, may be less than that in either tone delivered alone. A phenomenon of this sort is seen in the cochlear microphonic. Wever, Bray & Lawrence (1940) and Engerbretson & Eldredge (1968) showed that the amplitude of the cochlear microphonic was reduced by the simultaneous delivery of a second stimulus. The reduction was most prominent when the level of the second tone was much greater than the level of the first, which is usually the case during two-tone inhibition. It can be demonstrated that a phenomenon of this type is compatible with 'onset and termination', 'inhibition by a non-excitatory and

excitatory tone bursts', 'no inhibition of spontaneous discharges', and 'non-monotonic inhibition'. 'Adaptation and rebound' can be explained as characteristics of primary auditory neurones, because their time courses are similar to those of post-excitatory inhibition and the adaptation of responses to a tone burst, respectively. Although the cochlear microphonic shows a phenomenon comparable to two-tone inhibition, as far as its amplitude is concerned, it is not known to us whether or not 'phase shift' and 'latency difference' also exist in the cochlear microphonic.

Neural interaction

It has been established that two-tone inhibition is not mediated by the olivo-cochlear bundle, because it is evoked after the isolation of the eighth nerve from the medulla (Nomoto *et al.* 1964; Kiang, 1965). In addition, the time course of two-tone inhibition differs from that of inhibition evoked by the olivo-cochlear fibres (Fex, 1962) and from that of 'lateral inhibition' in other sensory systems (Ratliff, Hartline & Miller, 1963). Furthermore, we know of no histological reports of synapses between dendrites of primary auditory neurones. It has been pointed out, on the other hand, that these non-myelinated dendrites are apposed at the periphery and at the habenula perforata (Smith & Dempsey, 1957; Smith, 1967). Therefore, if two-tone inhibition involves neural interaction, it may be electrically, rather than chemically, mediated.

At least three possibilities for electrotonic interaction are conceivable: (1) interaction of receptor currents, (2) interaction of generator currents or (3) interaction of action currents. Since the excitation transmission from the hair cells to afferent nerve terminals appears to be chemical (e.g. Wersäll, Flock & Lundquist, 1965), the generator current is probably half-wave rectified, and thus different from the receptor current. The compound period histograms during inhibition were matched by adding the wave forms of the continuous tone and tone burst, as already described. The attempt was unsuccessfully made to match the histograms with the algebraic sum and the algebraic difference of the half-wave rectified wave forms of both. The interaction of action currents, of course, would differ depending on where the action potential is first initiated and where the action currents interact. If the action potential is evoked by a half-wave rectified generator current, the compound period histograms could not be matched by the sum of the original two wave forms. This is contrary to our results. Therefore, it may be suggested that two-tone inhibition is associated with a mechanism more peripheral than the point of rectification. A shunting model of electronic interaction of the generator current has been proposed (Furman & Frishkopf, 1964), but it was not supported by a quantitative analysis (Sachs, 1969). Furthermore, 'non-monotonic

inhibition', 'inhibition by an excitatory tone burst', and 'no inhibition of spontaneous discharges' are not explained by the shunting model.

Functional significance of inhibition

At higher levels of the auditory nervous system, inhibition of responses to a test tone burst can be evoked by the delivery of a preceding conditioning one, as well as by their simultaneous delivery (Suga, 1965*a*, *b*, 1968). But for the primary auditory neurones the two tones must be presented at the same time to produce inhibition. Therefore, in primary auditory neurones, parameters of the instantaneous structure of complex stimuli are more significant than parameters associated with preceding events. Since the discharge rate evoked by one tone can be increased or reduced as the amplitude of the second tone is increased, depending on whether or not the second tone is in an excitatory or inhibitory area, the number of discharges of a single neurone per cycle of the complex stimulus does not directly convey information about the stimulus amplitude. When the stimulus contains frequencies below about 4 kc/s, compound period histograms show that even during inhibition single neurones carry information about the frequency of both components. Although the neurones also carry phase information during inhibition, the phase relation between the two tones is different from that indicated by the compound period histograms of responses evoked by the individual tones.

As a phenomenon, two-tone inhibition is *qualitatively* similar to 'lateral inhibition', which has been found in various types of sensory systems of many animals (see Békésy, 1967). Lateral inhibition has been explained in terms of neural interaction through axon collaterals or inhibitory interneurones or both, which are not found in the cochlea. Two-tone inhibition may be due to a mechanism which differs from the lateral inhibition found in other sensory systems.

We wish to express our appreciation for the experimental assistance of Kay Williams, Mary Ann Mallon, and Ronald Cox and for the kind arrangements by Dr J. W. Hopkins, III, which made this work possible. This investigation was conducted under National Science Foundation research grant GB-13904 and the following support from the National Institutes of Health: Health Sciences Advancement Award (FR-504), Biomedical Sciences Support Grant (FR-07054) and Research grant NS-07498.

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