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Responses to Tones of Single Cells in Nucleus Magnocellularis and Nucleus Angularis of the Redwing Blackbird (*Agelaius phoeniceus*)

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Summary. Responses to tones were recorded from single cells in the brainstem auditory nuclei n. magnocellularis and n. angularis of the redwing blackbird (*Agelaius phoeniceus*). Whereas 30% of the cells in angularis had rates of spontaneous activity less than 40/s, only 5% of those in magnocellularis had rates this low. Two types of response maps are found in n. angularis. Type III maps have a single excitatory area with regions of inhibition on one or both sides. Type IV maps have a region of excitation at sound levels near threshold at the best frequency of the cell; at higher levels the best frequency is inhibitory; there can be broad bands of inhibition or interleaved bands of inhibition and excitation at higher levels. Cells in magnocellularis show only Type III response maps with generally weaker inhibitory sidebands than those in angularis. Responses to excitatory tone bursts in magnocellularis are "primary-like" in that they show a rapid increase in discharge rate at tone onset followed by a decrease in rate of 30 to 70% over the next 50 ms. On the other hand, a number of response types are seen in angularis, including "primary-like", "pauser", and "onset". These results for magnocellularis and angularis are compared with results from their presumed mammalian homologues, the anteroventral cochlear nucleus, and posteroventral and dorsal cochlear nuclei, respectively. Finally, the relationship between response patterns in these avian brainstem nuclei and those seen in higher auditory centers is considered.

Introduction

Interest in the avian auditory system has recently increased for a number of reasons. First, studies of vocal communication in birds have shown them to be appealing subjects for studies of the neurophysiological mechanisms underlying acoustic communications (Konishi, 1970). Second, new techniques have been developed which allow laboratory studies of auditory capacities of birds under operant conditioning paradigms (Dooling and Saunders, 1975; Hienz et al., 1977). Third, birds have become perhaps the most commonly used non-human species in studies of the ontogeny of vocal communication (Marler, 1970; Marler and Waser, 1977; Marler et al., 1972). Finally, birds have been used extensively in recent neurophysiological (Rubel and Parks, 1975), neuroanatomic (Rubel et al., 1976), and behavioral (Woolf et al., 1976; Gottlieb, 1971; Rubel and Rosenthal, 1975; Vince, 1969, 1973) studies of pre- and post-natal development of the auditory system.

Our long-term interest in the avian auditory system concerns the encoding of species-specific vocalizations in the discharge patterns of single neurons. Because such sounds must contain elements of behavioral importance to the organism, they form an especially appealing set of stimuli with which to probe the nervous system. Several recent studies with species-specific vocalizations have demonstrated apparently complex properties in the stimulus-response relationships of neurons at the higher levels of the avian auditory pathway; for example, in nucleus mesencephalicus lateralis, pars dorsalis (MLD) (Scheich et al., 1977), and Field L (Leppelsack and Vogt, 1976). However, relatively little is known about stimulus processing at lower levels of the avian auditory system. Studies of the more peripheral parts of the auditory system are essential to our understanding of the mechanisms which lead to the properties observed at higher levels. In a previous study (Sachs et al., 1974) we have shown that stimulus encoding in the avian auditory nerve is quite similar to that in mammals. The purpose of the study here is to consider responses to pure tones of cells in two avian brainstem auditory nuclei, nucleus magnocellularis and nucleus

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angularis. Nucleus magnocellularis is thought to be homologous with the anterior ventral cochlear nucleus in mammals; nucleus angularis is thought to correspond with the dorsal and posteroventral cochlear nuclei (Boord and Rasmussen, 1963; Boord, 1969). We have studied both the temporal properties of responses of cells in these nuclei to tone burst stimuli as well as the organization of response- and inhibitory-areas in the frequency-pressure plane. In this paper, we shall compare the properties of cells in nucleus magnocellularis with those in nucleus angularis, and will also compare the response properties of these avian nuclei with the corresponding mammalian nuclei.

The experimental animal in this study is the redwing blackbird. This species is the subject of a long-term study of the neural encoding of avian vocalizations (Hienz et al., 1977; Sachs et al., in press).

Methods

Preparation

Adult male redwing blackbirds were obtained from the Patuxent Wildlife Center, Laurel, Maryland. The animals were anesthetized with chloralose (50 mg/kg, freshly dissolved in saline; supplementary doses were given as needed). After removal of the skin and musculature from the top of the skull, the skull was attached with dental cement to a head- and ear-phone-holder described previously (Goldstein et al., 1968). Most of the skull covering the cerebellum was removed. A large part of the cerebellum was removed by aspiration to expose the floor of the fourth ventricle. With this exposure, microelectrodes can be inserted directly into nucleus magnocellularis and nucleus angularis under microscopic observation. A platinum-iridium electrode, usually with a platinum black ball on its tip, was placed over the nucleus of interest. The microelectrode was advanced by a hydraulically coupled microelectrode drive from outside the sound-isolated chamber (Industrial Acoustics Co., Type 1204-A) in which the bird was located. As the electrode was advanced, noise bursts were presented to aid in finding single units.

Because of difficulty in locating electrode tracks (see below), typically only two or three tracks were made in each animal. Recording sessions generally lasted 10 to 12 h. During this time, no change in auditory sensitivity was observed.

Acoustic Stimuli

A Koss model Pro 4AA dynamic earphone was used as the acoustic signal source. The earphone was coupled directly to the external meatus. A calibrated probe tube leading to a Bruel & Kjaer $^1/_2''$ microphone was placed at the mouth of the acoustic coupler to allow calibration of the frequency-dependent sound pressure level near the tympanum. Such calibrations were done in every animal for which data are presented here. All sound levels specified in this paper are expressed as dB re 0.0002 dynes/cm^2, at the mouth of the coupler.

Sound stimuli were presented as 200 ms bursts with 10 ms rise- and fall-time. They were repeated once per second.

Protocol for Studying Units

Presentation of stimuli and data acquisition were under computer control. Times of occurrence of action potentials were recorded with respect to the onset of the stimuli and recorded on digital magnetic tape for off-line analysis. On-line dot displays allowed monitoring of the responses during data acquisition. In most cases, the following protocol was used once a unit was satisfactorily isolated.

1) Spontaneous activity: Interspike intervals were recorded until a total of 2272 spikes were collected, or until a total time of 3 min elapsed, whichever occurred first.

2) Probe tone analysis: to test the unit's sensitivity to sinusoidal stimuli, a series of 100 tones covering the range of 100 Hz to 10 kHz in evenly spaced linear increments were presented. The tones were presented sequentially, beginning at the lowest frequency and proceeding to the highest. The first series of these probe tones were presented at a moderate sound level (about 50 dB SPL). The level was then decreased in 10 or 20 dB steps until there was no indication of response at any frequency on the dot display. Often one or two series were presented at higher sound levels (70–100 dB SPL), especially if the unit appeared to have a high threshold. From these probe data, off-line plots of discharge rate versus frequency were computed.

The probe analysis technique allows us to sample responses with a fine frequency resolution. The rate versus frequency plots proved useful in displaying the general features of response variations with frequency. For example, the probe analysis usually provided enough information about the unit to specify its best frequency and presence of inhibitory areas at, above, or below best frequency. (Best frequency refers to the frequency at which threshold of response is lowest.) However, because only one stimulus presentation is available at each frequency, the resulting data are too noisy for quantitative analysis. We will show plots from probe analysis data only where appropriate to graphically emphasize points established with the more detailed methods discussed below.

3) Best frequency response analysis: A series of 200 ms tone bursts at the unit's best frequency were presented at a moderate sound level (about 50 dB SPL) and at a rate of 1/s. These data were used to construct PST histograms of the unit's responses to best-frequency tones.

4) Response map: After these analyses were completed, an attempt was made to obtain a detailed response map for the unit. These maps were generated in the following manner: Tones were presented at the unit's best frequency at each of 6 to 10 sound levels. At each level the stimulus was repeated 20 to 100 times (usually 25 times). This procedure was then repeated for frequencies below and above the best frequency in steps of $^1/_8$ or $^1/_4$ octave. The process was continued until either the unit's entire response area was covered or the unit was lost. Off-line, post-stimulus time (PST) histograms were plotted from the stored data. These histograms were used to construct a response map which shows, as a function of frequency and sound pressure level, where the unit was excited and inhibited (see Young and Brownell, 1976). The ratio of perstimulatory rate (number of spikes per second occurring from the start of signal onset to the start of signal termination) to spontaneous rate (number of spikes per second occurring during the last 100 ms of the interstimulus interval) was computed. Excitatory regions were defined as areas where this ratio exceeded 1.25; inhibitory regions were those where the ratio was less than 0.75. If adjacent points showed different response types, that is, one was excitatory and one inhibitory, the ratio was interpolated between the points to define a single boundary between excitatory or inhibitory areas. Boundaries of excitatory and inhibitory areas were drawn by connecting these points. Similarly, boundaries are drawn between regions of inhibition or excitation and regions of

no response. Clearly the boundaries are not abrupt, as can be seen from the probe analysis plots in Figures 4, 11 and 12. Drawing a single boundary is simply a graphic convenience. In all plots, excitatory regions are shown as shaded areas; inhibitory regions are unshaded. All response maps shown here were constructed in this manner and not using probe analysis data.

Histology

Early in this series of experiments, location of electrode tracks proved very difficult. As a result, in almost all animals only a single electrode was used in nucleus magnocellularis or nucleus angularis. The locations of all tracks relative to one another were carefully recorded. On the last track a series of two to four lesions were made by passing current (20 μA for 20 s, electrode negative) through the electrode. One lesion was always made at the center of the range (in depth) over which auditory units were recorded. At the end of each experiment the animal's head was removed and placed in 10% buffered formalin for at least two days. Most of the skull was then removed from the brain. Some bone was left around the cochlea and brainstem. The remaining skull was then decalcified in equal parts of 10% EDTA in 0.1 M TRIS and 10% formalin. The decalcification proved necessary to avoid damage to nucleus angularis during removal of the brain from the skull. The brainstem and cochleae were then embedded in celloidon. Frontal sections were cut at 35 μm intervals and stained with the Kluver-Barrera method (Margolis and Pickett, 1956). The lesions were clearly visible in these sections and showed clearly whether the track had passed through nucleus magnocellularis or nucleus angularis. The position of a unit was localized along the track according to the reading of the electrode micrometer when the unit was studied. Corrections for tissue shrinkage were made using the micrometer settings at points where different lesions were made. With any electrode only the anterior/posterior or medial/lateral position, not the vertical angle, was changed from track to track. The position and orientation of each track could then be obtained relative to the track with lesions. Unit locations along such tracks were found from the micrometer reading of the unit and the micrometer reading for the brain surface on that track. Data are presented here only for units which could be unequivocally assigned to either nucleus magnocellularis or nucleus angularis.

Results

Spontaneous Activity

Spontaneous activity was measured in 88 units from nucleus angularis and 61 units in nucelus magnocellularis. Figure 1 shows histograms of the rates of spontaneous activity for units from the two nuclei. The primary difference illustrated by this figure is that the mode of the histogram for angularis occurs at rates less than 10/s, whereas no magnocellularis units had rates less than 10/s. Thirty percent of the angularis units had rates less than 40/s; only 5% of magnocellularis units had rates this low. The mean rate for magnocellularis was 115.7 spikes per second with a range of 16 to 237 spikes per second. In angularis the mean rate was 45.9 spikes per second with a range of 0 to 174 spikes per second.

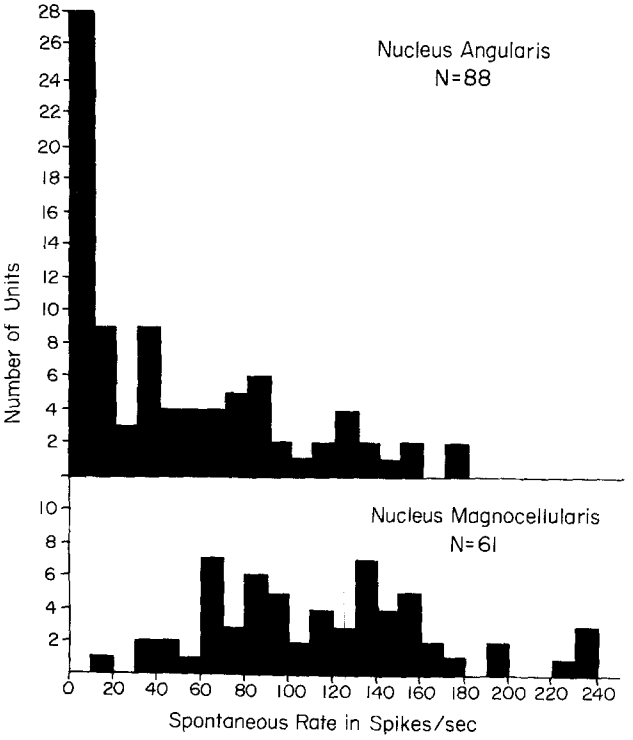

Fig. 1. Histograms showing number of units with various rates of spontaneous activity. The height of each bar in the histogram equals the number of units with spontaneous rates within 10 spikes/s of the rate at the left of the bar. Data from 88 nucleus angularis units and 61 magnocellularis units

Figure 2 compares the time patterns of spontaneous activity of cells in nucleus magnocellularis and nucleus angularis. The time patterns of spontaneous activity are most often displayed in terms of interspike-interval histograms. A statistic useful in describing the shapes of such interval distributions is the coefficient of variation, defined as the ratio of the standard deviation to the mean of the interval distribution. For example, Goldberg and Brownell (1973) have shown that the coefficient of variation is related to the symmetry of interval histograms and to the mode/mean ratio. Small coefficients of variation correspond to symmetric interval distributions with high mode/mean ratios. Figure 2 shows the distributions of coefficients of variation across cells in nucleus magnocellularis and nucleus angularis, respectively. For both groups, interspike-interval histograms representative of the high- and low-ends of the distribution of coefficients of variation are also shown. The mean c.v. in magnocellularis is slightly greater than that in angularis (0.89 vs. 0.84). This difference reflects both the larger number of units with c.v. near one in magnocellularis and the fact that no magnocellu-

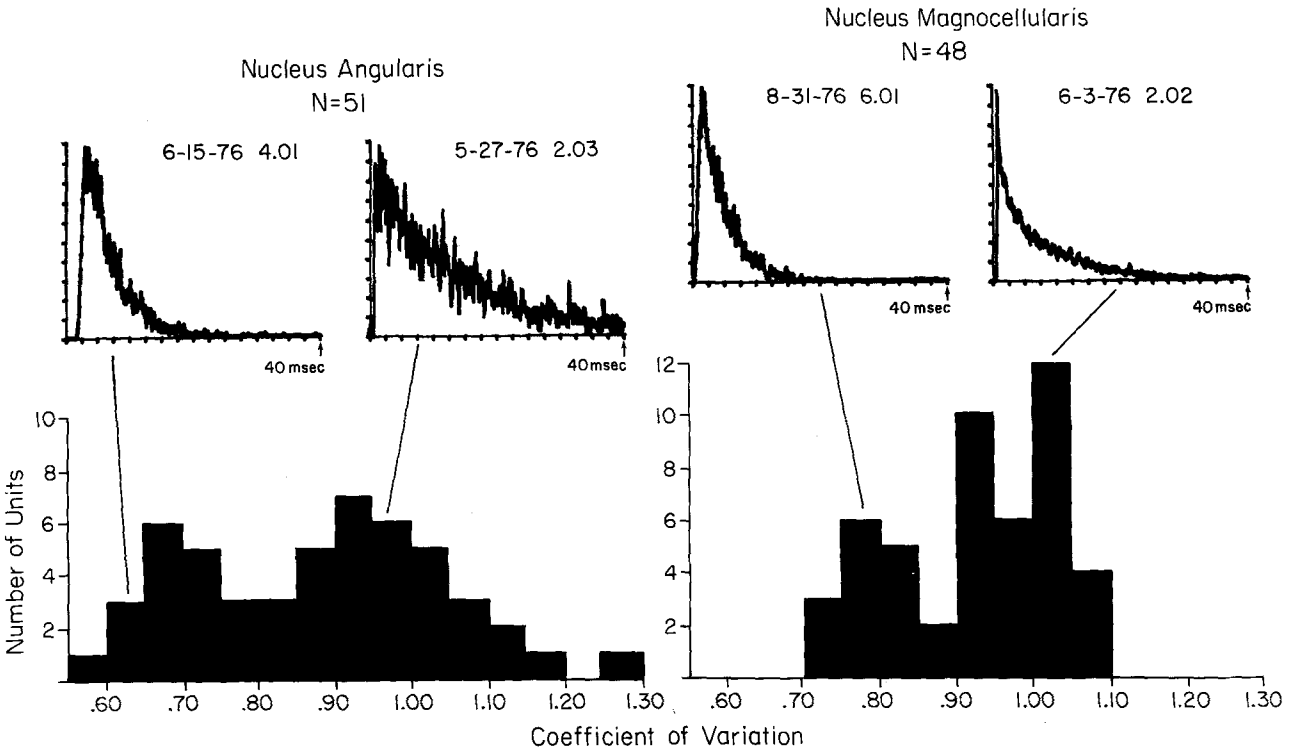

Fig. 2. Histograms showing number of units with various coefficients of variation (CV), defined as the ratio of the standard deviation to the mean of the interspike interval distribution. The height of each bar in the histogram equals the number of units with CVs within 0.05 of the value shown to the left of the bar. Insets show representative interspike interval distributions, corresponding to CVs at the upper and lower ends of the scale. Each separate unit has a label for identification purposes, e.g., 6–15–76 4.01 represents animal number (6–15–76), track (4), and unit number (01) obtained in that track

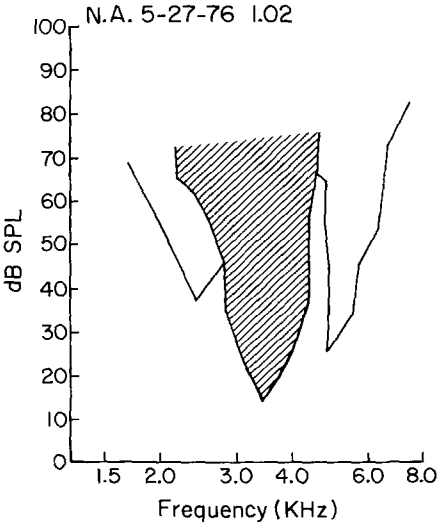

Fig. 3. Response map for a type III unit from nucleus angularis. Excitatory regions are shaded, inhibitory regions are unshaded areas enclosed with solid lines

laris cells have c.v.'s less than 0.7, while 10 angularis cells have c.v.'s between 0.55 and 0.70. Nonetheless, the data in Figure 2 are perhaps best summarized by pointing out that there is great overlap in the distribution of c.v.'s between these two nuclei, and thus the corresponding time patterns of activity differ very little.

Response Maps

Nucleus Angularis. Reasonably complete response maps were obtained from 45 units in nucleus angularis as described above. In addition, sufficient information was obtained from the probe technique to classify the response maps of 70 additional units from angularis, giving a total sample of 115 units. Two types of response maps are found in nucleus angularis, which differ from one another in the distribution of regions of inhibitory responses in the frequency-sound level space. Of the units in our sample from nucleus angularis, 28 had spontaneous discharge rates of less than 10/s. For these units we cannot say anything about the distribution of inhibition in the frequency-level space. The most common type of response map for units with spontaneous rates greater than 10/s is illustrated in Figure 3. A total of 76 units showed the type of response map illustrated in Figure 3. Response maps of this type have a central V-shaped excitatory region (shaded region). Inhibitory re-

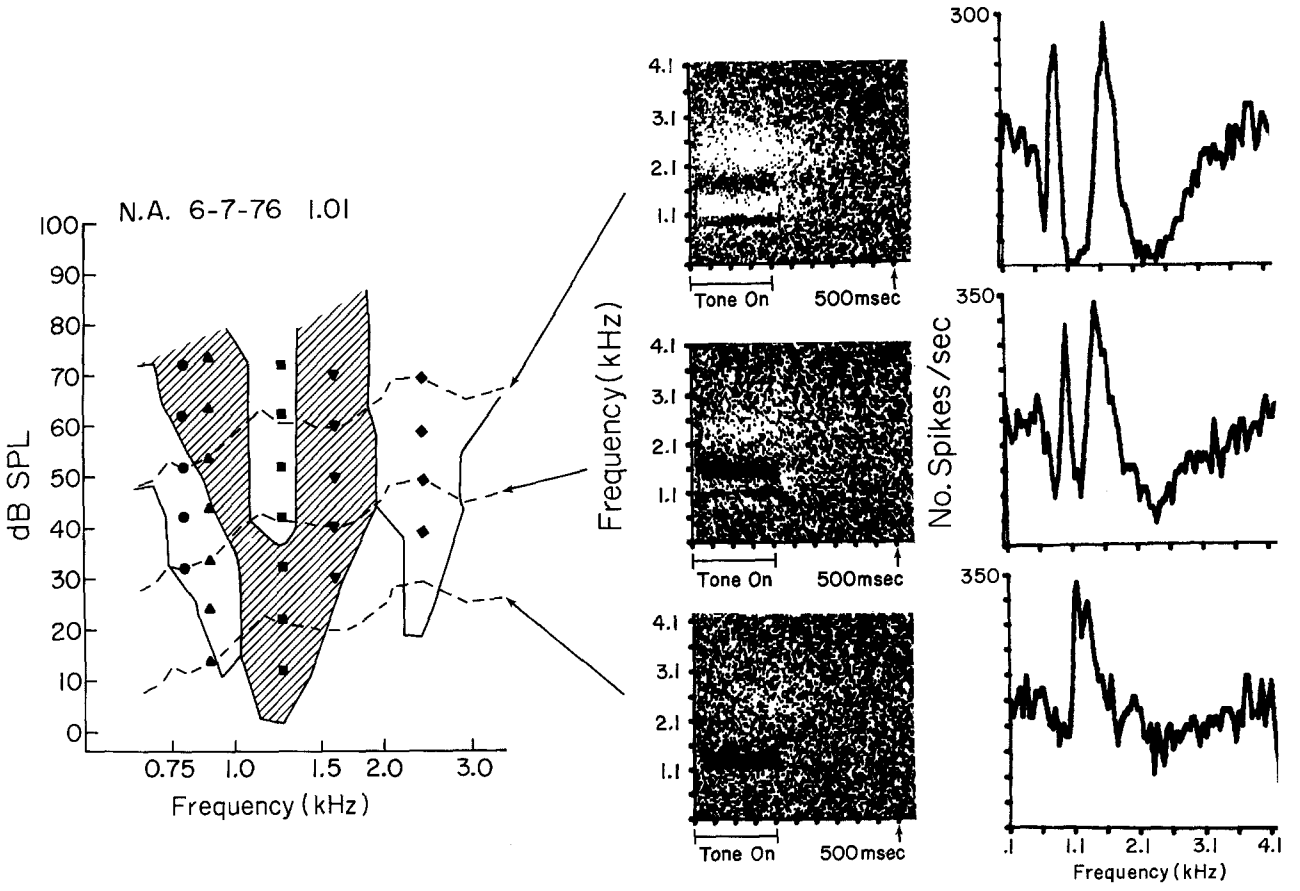

Fig. 4. Response map for a type IV unit from nucleus angularis is shown on left. At center are dot displays showing responses to probe tones at three different levels. Each line in the display gives responses to the frequency given by the ordinate. For each dot display, the voltage into the earphone is constant, but the resulting acoustic calibration is not flat and is indicated by a dashed line in the response map. Arrows point to plots of sound level at the eardrum versus frequency for the corresponding earphone voltage. The calibration plots are helpful in relating the probe analysis data to the detailed response map made with a different technique. At right are shown the discharge rate versus frequency plots for the probe analysis data. On the response map, symbols correspond to the stimulus levels for the histograms shown in Figure 10

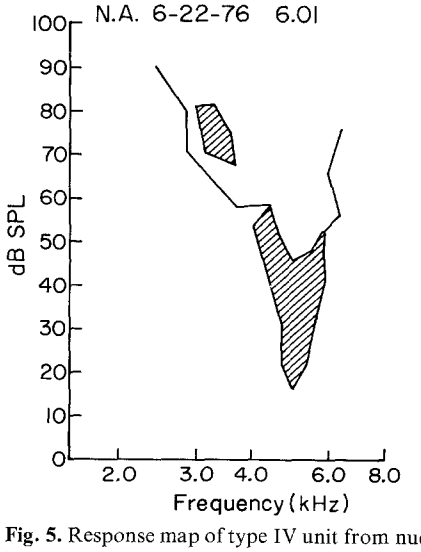

Fig. 5. Response map of type IV unit from nucleus angularis, showing an excitatory region isolated from the excitatory region near best frequency

sponses were found in the surrounding regions enclosed by solid lines. Notice that for this type of response map, tones at the unit's best frequency are excitatory at all levels above thresholds.

A second type of response map, shown in Figure 4, was found in 11 units in nucleus angularis. Complete response maps were obtained from all of these units. The detailed organization of the response map for this type varied considerably from unit to unit. One aspect is consistent across all units with this type of map, however. As illustrated in both the probe analysis data and in the detailed response map, tones at the unit's best frequency are excitatory at sound levels near threshold but are always inhibitory at higher levels. Inhibitory regions at higher stimulus levels extended to frequencies both above and below best frequency. Excitatory areas were also observed at higher levels. In some cases these areas were continuous with the excitatory area at best fre-

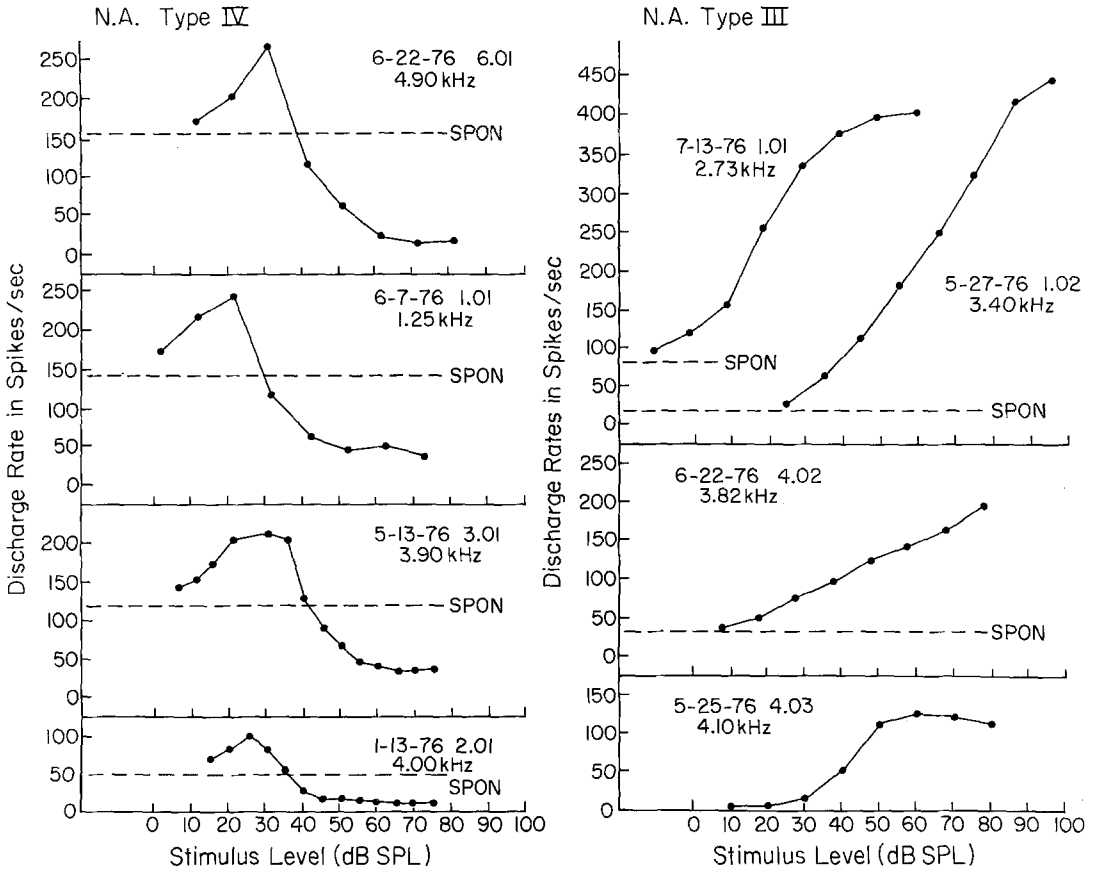

Fig. 6. Discharge rate versus sound level functions for 8 nucleus angularis units responding to best frequency tones. Units on the left are type IV, those on the right are type III. Dashed lines show rates of spontaneous activity

quency, as in Figure 4; in other cases the excitatory areas at higher levels were isolated from the excitatory area near best frequency, as in Figure 5. The resulting interleaved arrangement of excitatory and inhibitory response areas can be quite complex; at a fixed sound level above threshold the response of a unit can change from inhibitory to excitatory and vice-versa several times as frequency is changed from low to high. (See, for example, the probe analysis plots in Fig. 4).

The primary difference between response maps like that in Figure 3 and those in Figures 4 and 5 is illustrated in Figure 6. Here we plot discharge rate versus stimulus level for best-frequency tones for 8 units from nucleus angularis. The stimuli in each case were 200 ms tone bursts at the best frequency of the unit. Discharge rate is averaged over the total duration of the 200 ms tone bursts. The response maps for units on the right were similar to that in Figure 3. For these units rate is a monotonic function of sound level for best frequency tones. The response maps for units on the left of Figure 6 are similar to those in Figures 4 and 5. For units with this type of response map, rate-level functions for best frequency tones are strongly nonmonotonic, with discharge rate decreasing to zero at high stimulus levels.

Nucleus Magnocellularis. We did not find such a variety of response maps for units in nucleus magnocellularis. All of the units in our sample of 66 had response maps similar to those shown in Figure 7. They are characterized by a roughly V-shaped excitatory region whose tip is at the best frequency of the unit. Inhibitory regions can occur one or both sides of the central excitatory region. Figure 7 shows the range of inhibitory areas observed in magnocellularis, from the most limited (left), to the broadest (right).

For convenience we shall call units with response maps like those in Figures 3 and 7 Type III. Response maps like those in Figures 4 and 5 will be called Type IV. In Discussion we will consider the relation of units so classified to units with similar classification in the mammalian cochlear nuclei (see Evans and Nelson, 1973; Young and Brownell, 1976).

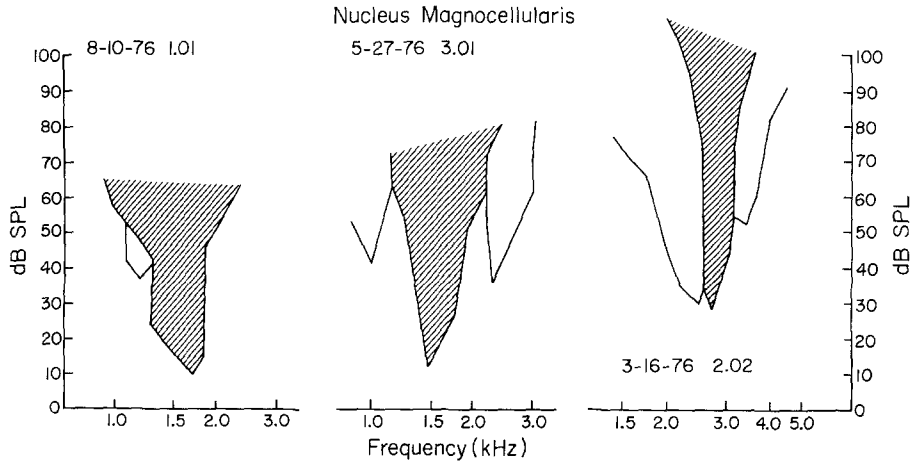

Fig. 7. Response maps for 3 units in nucleus magnocellularis. These show the range of inhibitory areas seen, from the most limited (left) to most extensive (right)

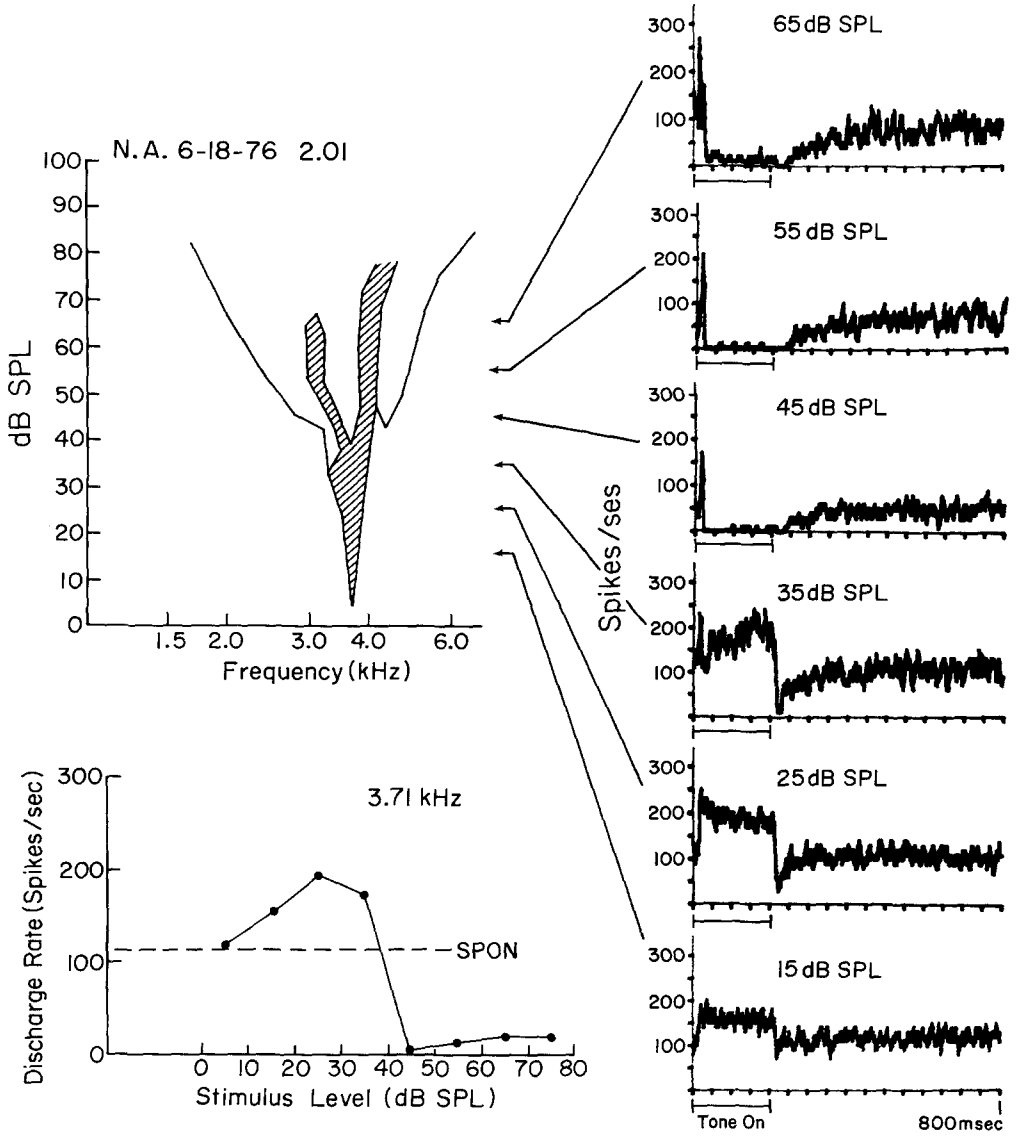

Fig. 8. Responses of a type IV unit to best frequency tones at various sound levels. The arrows associate each PST histogram with the corresponding stimulus level on the response map. The rate versus level function for these best frequency tones is also shown

Response Histograms

Best-Frequency Tones. The response maps discussed above provide information only about the behavior of discharge rate averaged over the duration of a tone burst. Details of how the response of a unit changes with time during the burst can be displayed in PST histograms. Figure 8 shows how the responses of one type IV unit from nucleus angularis change with sound level for tones at the unit's best frequency. The corresponding rate versus level function is shown below the response map. At 15 dB SPL (about 10 dB above unit threshold) the unit responds with an approximately constant discharge rate about 60% above its rate of spontaneous activity. There is little or no rate adaptation during the 200 ms of the burst. At 25 dB SPL the driven discharge rate has increased to about twice the spontaneous rate and rate adaptation is evident. At 35 dB SPL, there is an initial increase in discharge rate; this increase is followed by a brief decrease in rate and then a return to a rate approximately equal to the onset rate. At higher sound pressure levels, a short initial onset burst is followed by inhibition of spontaneous activity for the remainder of the burst. This variation in response patterns with sound level for best frequency tones is typical of the Type IV units in our sample. One feature seen in some of the Type IV units is not evidenced in this unit, however. As illustrated in Figure 10, for both best frequencies and other frequencies the perstimulatory inhibition can be followed by an after discharge.

The response patterns to best frequency tones of the Type III units in our angularis sample were similar to those observed in earlier studies of the mammalian cochlear nuclei (Pfeiffer, 1966; Godfrey et al., 1975). PST histograms of responses to 200 ms tone bursts are shown for three Type III angularis units in Figure 9. Three types of histograms were observed. A great majority of the sample (93 of 104 units) produced simple response histograms. Discharge rate rises rapidly at stimulus onset and then decays to a more or less steady level. Such response patterns have been called "primary-like" when observed in the mammalian cochlear nuclei (Pfeiffer, 1966). Of the remaining units in the sample, 5 showed response patterns to best frequency tones similar to that in the center of Figure 9. This response pattern has previously been called a "pauser" (Pfeiffer, 1966). In this pattern an initial onset discharge is followed by a rapid decline in firing rate and then a buildup to a more or less steady discharge rate. The remaining 6 units discharged one or two spikes at stimulus onset and were subsequently silent. A PST histogram for one so-called "on" unit is shown at the bottom of Figure 9.

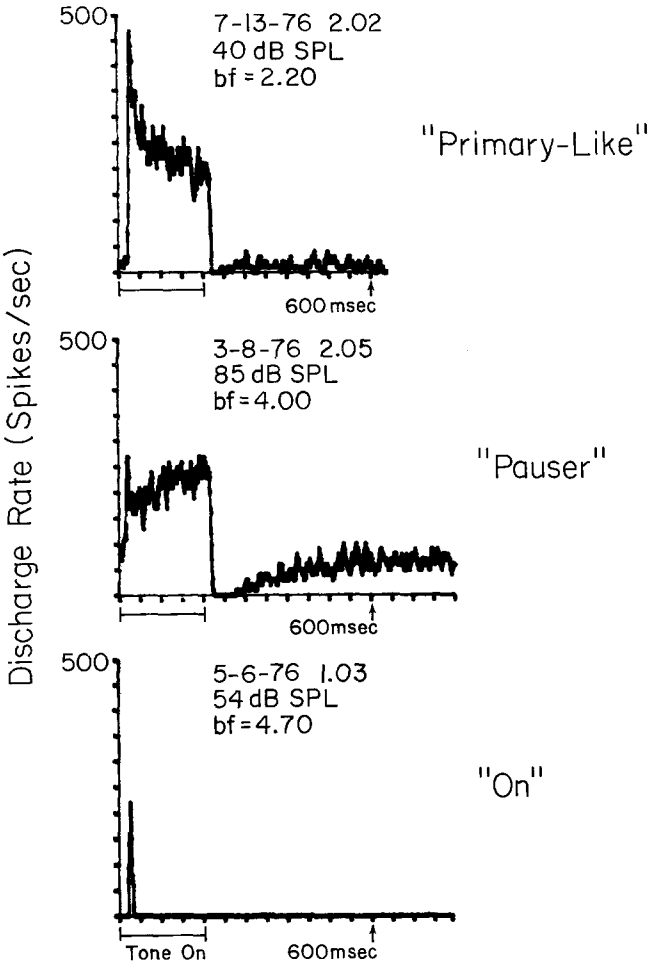

Fig. 9. PST histograms for best frequency tones from three type III units in nucleus angularis. These histograms represent the three types observed in this study

All of the 66 units studied in magnocellularis showed the "primary-like" response pattern (see, for example, Fig. 12).

Variation of Response Patterns with Frequency

The variation of response patterns with sound level can depend on stimulus frequency in a quite complicated way for units with "type IV" response maps. Figure 10 shows one example. The response map for the unit illustrated in this figure is shown in Figure 4. At best frequency, the response pattern changes from excitation at near-threshold levels to a predominantly inhibitory perstimulatory response at higher levels. At tone-off there is a brief burst of spikes not quite reaching the spontaneous rate and then a

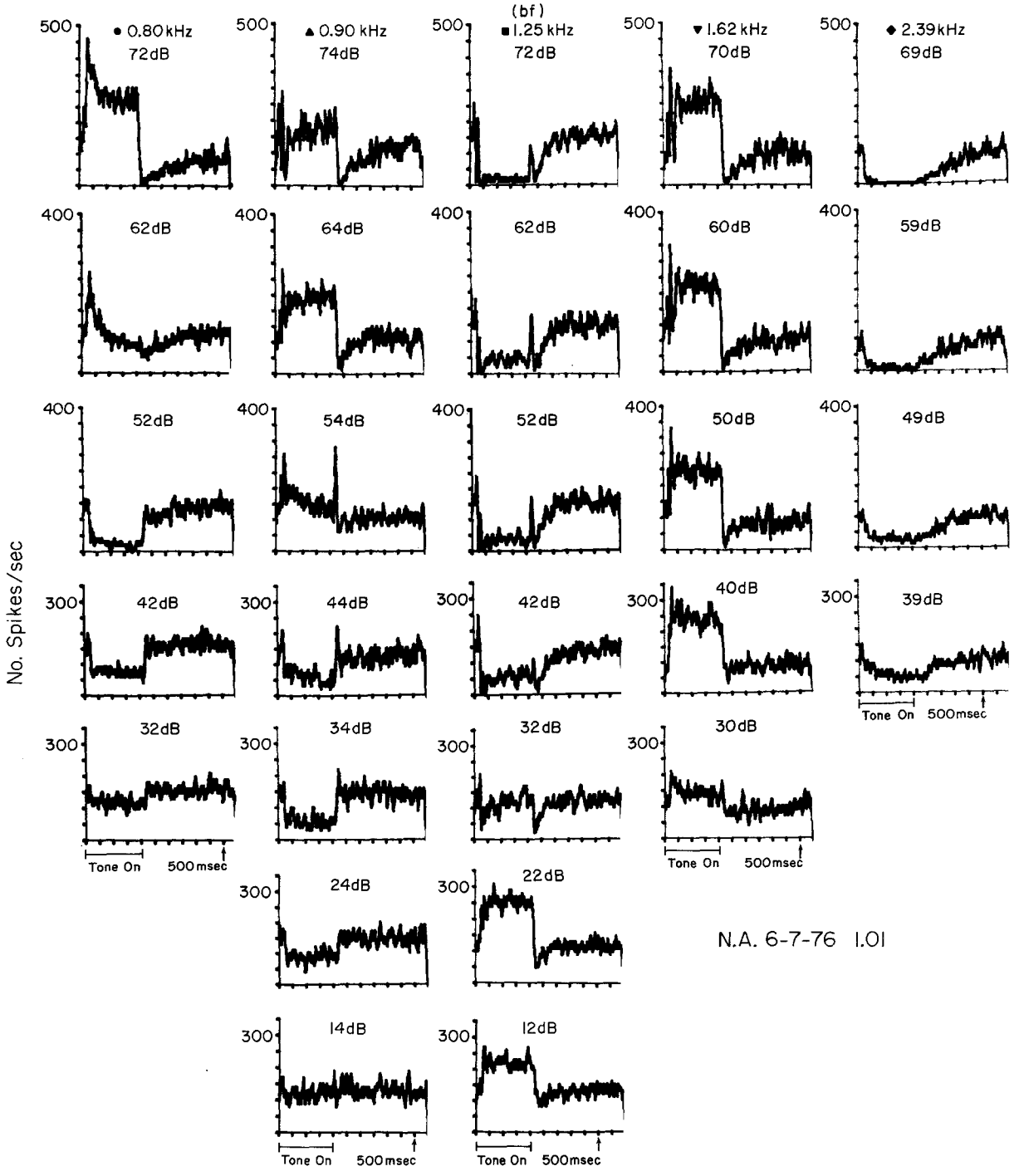

Fig. 10. Responses of a type IV unit to tones of various frequencies and sound levels. The response map for this unit is shown in Figure 4. Frequencies are given at the top of each column; the symbols correspond to those on the response map in Figure 4 where they indicate the stimulus conditions

gradual return to spontaneous. At frequencies just below best frequency the response pattern at low stimulus levels is inhibitory with both on- and off-bursts. At higher levels the pattern becomes similar to the "pauser" pattern shown in Figure 9. At lower frequencies the "pauser" response is not present at high levels. Furthermore, the after discharge is absent in the inhibitory responses. At frequencies just above the best frequency the response pattern progresses from "primary-like" at low levels to "pauser" at

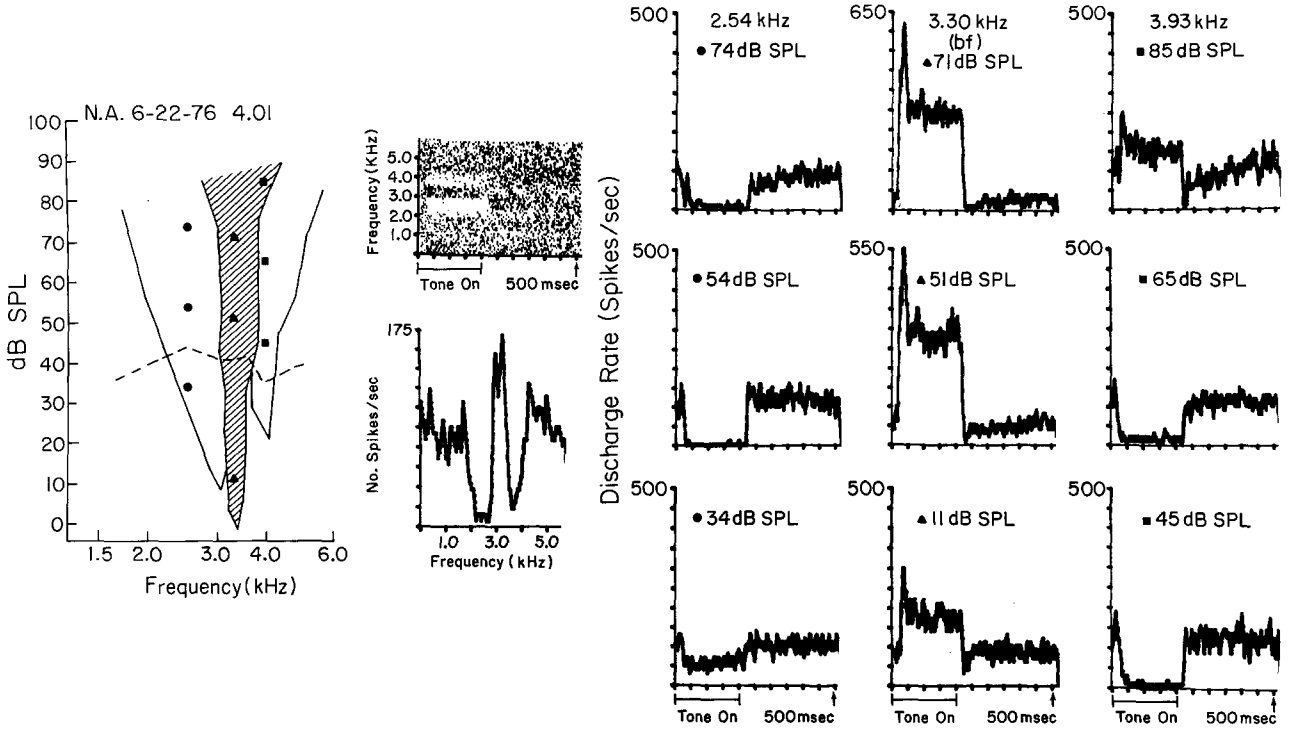

Fig. 11. Responses of a type III unit from nucleus angularis to various frequencies and sound levels. The positions of various stimuli are indicated by corresponding symbols on the response map. Rate versus frequency plots are from the probe analysis data shown

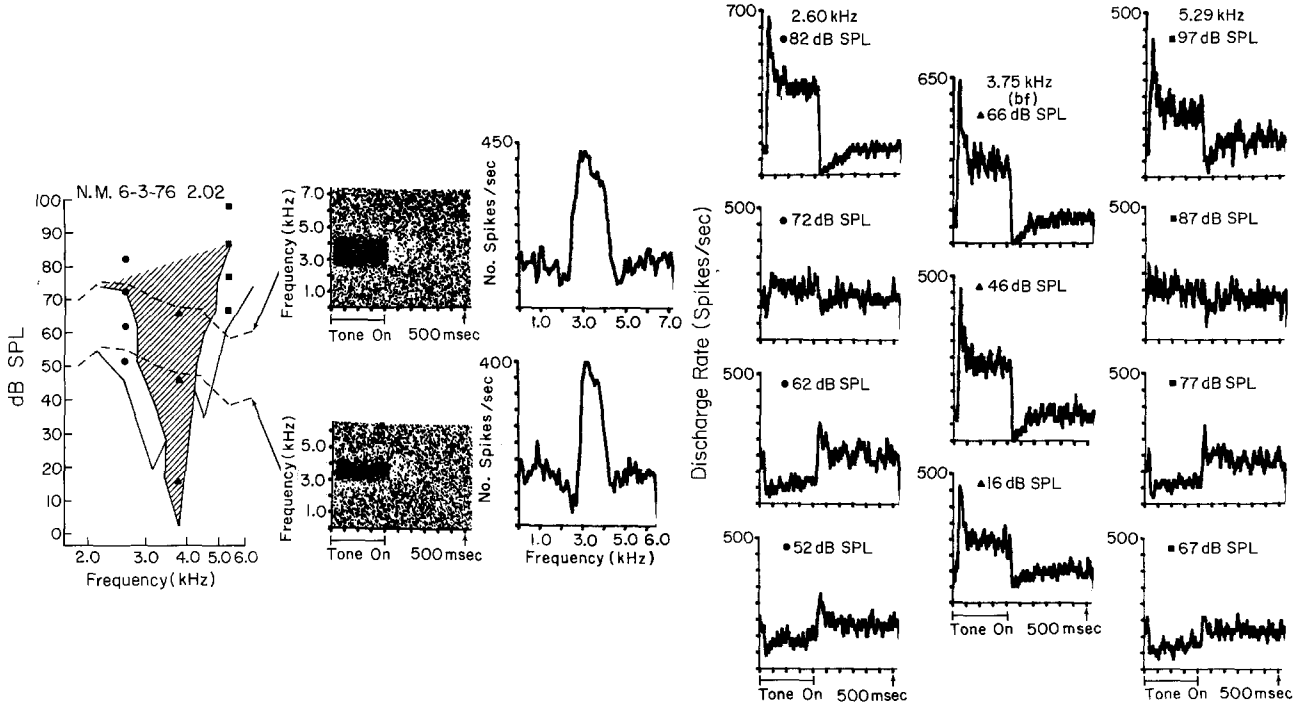

Fig. 12. Responses of a unit from nucleus magnocellularis to various frequencies and sound levels. The positions of various stimuli are indicated by the corresponding symbols on the response map. Rate versus frequency plots are from the probe analysis data shown

higher levels. At still higher frequencies the response pattern remains inhibitory at all levels tested. Notice that at frequencies off best frequency the pause in the pauser responses is much more distinct than at best frequency.

The response patterns of the "type III" nucleus angularis units are much simpler than those of the "type IV" units. As is illustrated in Figure 11 the pattern for frequencies near best frequency is excitatory at all sound levels. At frequencies below or above best frequency, the pattern is predominantly inhibitory at most levels, and becomes excitatory only at the highest levels used.

The variation in response pattern with frequency of units in nucleus magnocellularis is similar to that of the type III units in nucleus angularis. There is, however, a consistent difference which is illustrated by comparing the unit shown in Figure 12 with the type III unit of Figure 11. These two units are closely matched in spontaneous rate, best frequency and threshold. As is illustrated by both the PST histograms and the probe analysis data, the maximum inhibition for cells in magnocellularis is considerably less than that for angularis. Typically, discharge rate is suppressed to almost zero by some frequency-level combination for cells in angularis (e.g., Fig. 11). For cells in magnocellularis, on the other hand, rate is rarely maintained at less than 40% of the spontaneous rate, as illustrated in Figure 12, and the inhibitory areas for cells in magnocellularis can be quite weak as is shown in Figure 7.

Discussion

Comparison of Nucleus Angularis and Nucleus Magnocellularis: Summary of Results

A primary thrust of the studies described here has been to compare response properties of cells in nucleus angularis with those in nucleus magnocellularis. This comparison has been made along a number of dimensions and it will be useful for further discussion to summarize these briefly here.

1) Spontaneous activity: There are no units with rates of spontaneous activity less than 10/s in nucleus magnocellularis, whereas the mode of the spontaneous rate distribution for nucleus angularis is less than 10/s (see Fig. 1). Shapes of interval histograms are similar for the two nuclei, although histograms with the greatest symmetry are found in angularis (Fig. 2).

2) Response maps: Two types of response maps for single tones are found in nucleus angularis whereas only one type is found in magnocellularis. Type III response maps (nucleus angularis) have a single V-shaped excitatory area with regions of inhibition on one or both sides of the excitatory region (Figs. 3 and 11). Type IV maps (nucleus angularis) have a region of excitation at sound levels near threshold at the best frequency of the cell; at higher levels the best frequency is inhibitory. Furthermore, at higher sound levels there can be broad bands of inhibition or interleaved bands of excitation and inhibition (Figs. 4, 5 and 8). Cells in magnocellularis show only "type III" response maps (Figs. 7 and 13). In general, the inhibitory sidebands in magnocellularis are considerably weaker than those in angularis.

3) Response histograms: For all cells in our magnocellularis sample, response histograms to excitatory tones are characterized by a rapid increase in discharge rate at tone onset followed by a decrease in rate of 30 to 70% over the next 50 ms. Inhibitory tones cause a decrease in rate from spontaneous of less than 60% (Fig. 12). Patterns were more varied for cells with type III response maps in angularis. Three types of response histograms are seen; each of these can be seen at best frequency (Fig. 9). "Primary-like" response histograms are similar to those described for magnocellularis and were seen at best frequency in 89% of the units in our sample; 5% of the cells had "pauser" type responses which were characterized by an onset discharge, a pause in firing, then a rapid buildup to a steady discharge rate; 6 cells ("on" units) discharged only at tone onset. Responses to tones which are predominantly inhibitory in type III angularis units were similar to those in magnocellularis. However, the inhibition in angularis was typically stronger than that in magnocellularis; often spontaneous activity was almost completely extinguished (Fig. 11). Variations of response histograms with sound level and frequency for type IV units were quite complex.

We should indicate that there may be some bias in our sample of units from nucleus magnocellularis. In nucleus angularis the range of best frequencies found was from 0.35 to 7.0 kHz; 50% of the units had best frequencies greater than 3.7 kHz. In nucleus magnocellularis the range was 0.75 to 6.5 kHz, but only one cell had best frequency greater than 3.7 kHz.

Comparison with Previous Studies of the Avian Auditory System

The afferent auditory input to both nucleus magnocellularis and nucleus angularis is via the fibers of the auditory nerve. By comparing the response properties of cells in these two secondary nuclei with their primary input we can begin to understand the processing

which occurs at these early stages of the avian auditory system. There have been two studies of response properties of single fibers in the pigeon auditory nerve (Sachs et al., 1974; Gross and Anderson, 1976). Sachs et al. found that the responses of single auditory-nerve fibers in birds closely resemble those of mammals, with the exception that rates of discharge (both spontaneous and driven) were considerably higher in birds than in mammals. The high rates of spontaneous activity in nucleus magnocellularis (Fig. 1 of this paper) may thus be a reflection of the high rates seen in the auditory nerve (Fig. 1, Sachs et al., 1974). Further evidence of this relationship comes from studies of the cat anteroventral cochlear nucleus, thought to be homologous with nucleus magnocellularis. Koerber et al. (1966) have shown that the spontaneous activity of cells in the cat AVCN disappears when the cochlea is destroyed; they conclude that the spontaneous activity in these secondary cells is driven by the spontaneous activity in their primary afferent input. Sachs et al. (1974) did not find that spontaneous activity of auditory-nerve fibers was inhibited by single tones at the sound levels they used. However, their techniques for obtaining response areas were probably not sensitive enough to show the weak inhibition seen in most magnocellularis units. Gross and Anderson (1976) did find inhibitory responses in some auditory-nerve fibers. Recent studies in our own laboratory have shown that discharge rate of some auditory-nerve fibers in redwing blackbirds can be reduced by single tones to a rate less than that seen in the absence of controlled stimulus (Woolf and Sachs, 1977). Because of the high sensitivity of fibers in these birds, ambient noise (e.g., the animal's own sounds) cannot be ruled out as a source of some of this ongoing activity. In any event, we cannot rule out inhibition in the afferent input as the source of inhibitory sidebands found for cells in our nucleus magnocellularis sample. Sachs et al. (1974) reported that the response histograms of avian auditory-nerve fibers for tones were similar to those seen in cats and are similar to those observed in our mangocellularis population and the "primary-like" angularis responses. Gross and Anderson (1976) reported some tone responses in auditory-nerve fibers which were not "primary-like". The reasons for this discrepancy are not obvious.

There have been three previous studies of responses of cells in the avian cochlear nucleus. Konishi (1970) and Rubel and Parks (1975) concentrated their studies on the tonotopic organization of this nucleus. Konishi studied both magnocellularis and angularis in a number of species and presents the most detailed analysis of sparrows; Rubel and Parks studied hatchling chickens. The organization is similar in both studies. The cells in magnocellularis are arranged in dorso-ventral iso-frequency columns, with high frequencies located rostromedially and lower frequencies found at more caudal and lateral sites. In angularis, Konishi found that best frequencies increase in the medial to lateral, rostral to caudal and ventral to dorsal directions. Because we wanted to be able to locate all electrode tracks, we did not make enough passes in any one bird to precisely define the tonotopic organization of either nucleus angularis or nucleus magnocellularis. However, our results are consistent with these previous studies. Specifically, tracks in angularis showed a clear decrease in best frequency from dorsal to ventral, whereas there was very little change in best frequency with depth in nucleus magnocellularis.

Rubel and Parks (1975) report that cells in nucleus magnocellularis show "primary-like" response patterns, as was the case in the present study. They also report "clear evidence that spontaneous activity of many units could be inhibited by tonal stimuli." All of the magnocellularis units in the present study had inhibitory sidebands. Stopp and Whitefiled (1961) recorded from "100 units from auditory nuclei in the brainstem of pigeons, mostly from magnocellularis." Of these, approximatelly two-thirds could be inhibited by pure tones. In their studies, the inhibition was in some cases only partial even with quite strong stimuli. This finding of partial inhibition may correspond to our observation that for cells in magnocellularis discharge rate usually could not be reduced to less than 40% of the spontaneous rate by tonal stimuli. There is one aspect of the Stopp and Whitfield results which is quite different from our findings and those of Rubel and Parks (1975). Stopp and Whitfield report that 21 of the 100 units in their sample could be inhibited but not excited by sound stimuli. All of our magnocellularis units were excited by tones; in fact, even in our angularis sample only one unit was not apparently excited by any frequency or level which we tried. Two possible reasons for this discrepancy occur to us: there could, of course, be a species difference (pigeon versus blackbird); also, different anesthetics were used (urethane and chloralose); however, as we shall point out below, it seems likely that chloralose would enhance the opportunity to find inhibitory units.

Scheich and his collaborators (1977) have recently been analyzing the response of cells in nucleus mesencephalicus lateralis pars dorsalis (MLD) to species-specific vocalizations. Their experimental animal is the guinea fowl. Although MLD receives a variety of inputs, at least some cells in nucleus angularis project directly to MLD (Boord, 1969). It is appropriate, therefore, to relate our results to those of Scheich

et al. They classify neurons roughly into two classes. Their "simple" neurons have response maps similar to those of our type III units. For these neurons, responses to calls were predictable from the call spectra and the unit's response map. For what they called "complex units", on the other hand, responses to calls were not easily predicted from the call spectra and the unit's response map. Some of their "complex" neurons were characterized by rate-versus-frequency plots similar to those of our type IV units as illustrated by the probe analysis in Figure 4. That is, they had at least four bands of inhibition and excitation; or they could have wide inhibitory bands, similar to our Figure 5. Thus, it appears likely that some of the properties of the "complex" cells observed in MLD can be directly related to properties of cells in nucleus angularis. It is important to point out here, however, one very important difference between our results and those of Scheich et al. (1977). In the Scheich study, 50% or more of the cells had these complex properties. Only 10% of our population of nucleus angularis cells were type IV. One possible reason for this difference might be that the Scheich experiments were all done with unanesthetized animals, while our animals are chloralose anesthetized. As we elaborate below, anesthesia can affect type IV response maps. Furthermore, it is certainly to be expected that further processing of auditory stimuli occurs at the MLD level as well as at intermediate levels (e.g., superior olive, nucleus of the lateral lemniscus).

Comparison with Studies of Mammalian Cochlear Nuclei

There have been a number of papers which have described response maps of cells in the cochlear nuclei of cats (Greenwood and Maruyama, 1965; Goldberg and Brownell, 1973; Evans and Nelson, 1973; Young and Brownell, 1976). Both Evans and Nelson (1973) and Young and Brownell (1976) have used unanesthetized and chloralose anesthetized preparations. Both have studied the effects of anesthesia on response maps in the dorsal cochlear nucleus (DCN). Evans and Nelson conclude that "the distribution (of response maps) obtained in DCN under chloralose anesthesia (Fig. 11c) approximates more closely to that in the unanesthetized state (Fig. 11d and e) than to that under halothane or pentobarbitone anesthesia (Fig. 11a and b)." It was this similarity between unanesthetized and chloralose anesthetized preparations which influenced our choice of chloralose anesthesia in the present study. Both of these cat studies showed that inhibitory responses could be changed into excitatory responses by administration of barbiturate anesthesia. Indeed, in two n. angularis experiments we first found a type IV response and immediately injected pentobarbital anesthesia. The inhibitory responses at best frequency which characterized the type IV unit turned into excitatory responses within 15 s of administration of the anesthesia.

Evans and Nelson (1973) described five types of units in the dorsal cochlear nucleus. Their type I and type II units showed only excitatory responses to tones. The differences between the type I and II depended upon whether or not spontaneous activity was suppressed following tonal stimulation. The only units we found in nucleus angularis which did not have inhibitory areas in their response maps, had little or no spontaneous activity. The type III units of Evans and Nelson (1973) had response maps similar to those we have called type III. Young and Brownell (1976) did not distinguish between type I, II, and III units because 43% of their units of these types had no spontaneous activity. They combined these into one category which they called type II/III. Both of these cat studies described type IV units as those with broad ranges of inhibition and specifically inhibition by best frequency tones. For most of the type IV units in the Young and Brownell study there was a small region of excitation near threshold at the unit's characteristic frequency. Evans and Nelson did not report such excitation at best frequency. Evans and Nelson also described units which were only inhibited by tones (their type V units).

In the present study we have described units in nucleus angularis with response maps similar to those of type III and IV in the cat cochlear nucleus. It is difficult, however, to compare the proportions of types of units found in our bird studies and those found in cats. As pointed out above, nucleus angularis is thought to be homologous with both DCN and PVCN (Boord and Rasmussen, 1963; Boord, 1969). The medial subdivision of angularis is probably homologous with PVCN, while the lateral division is homologous with DCN. Our unit recordings came from tracks which were almost equally divided between the lateral and medial divisions of nucleus angularis. All of the 11 type IV units in our sample came from the lateral third of nucleus angularis, which would place them in the homologue of DCN. Type IV units have only been found in DCN in the cat.

All of the units in our magnocellularis sample had inhibitory sidebands, whereas only about 20% of the units in chloralose anesthetized cat VCN showed inhibition (Evans and Nelson, 1973). In barbiturate anesthetized cats, Evans and Nelson found that less than 10% of units in the VCN show inhibition.

On the other hand, Brownell (1975) found that most of those axons of the trapezoid body which originate from globular cells in the AVCN of cats had inhibitory sidebands. Only one out of eight fibers originating from spherical cells had inhibitory sidebands. Goldberg and Brownell (1973) found that only 3 of 34 units recorded in the spherical cell region of the AVCN had such sidebands. In cat VCN then, it appears that inhibitory sidebands are common only for cells other than spherical cells. Cells in this spherical cell region are innervated predominantly by a few auditory nerve fibers, ending in a calycoid fashion (Osen, 1969). It might be expected that these cells would closely reflect their primary input and thus not show inhibitory sidebands. Boord and Rasmussen (1963) describe innervation patterns similar to those of the spherical cells for cells in the medial and lateral parts of nucleus magnocellularis. (See also, Jhaveri and Morest, 1977.) On the basis of the electrophysiological results from cats and the neuroanatomical similarity between cat spherical cell region and magnocellularis, even the weak inhibitory sidebands seen in magnocellularis are puzzling. Such sideband inhibition has been observed previously in magnocellularis by Stopp and Whitfield (1961) and Rubel and Parks (1975). As we pointed out above, although the question of whether or not spontaneous activity in avian auditory nerve fibers can be inhibited has not been resolved, there is some evidence of inhibition of spontaneous activity for some fibers. Inhibition of spontaneous activity in cat auditory-nerve fibers is rarely, if ever, seen (Sachs and Kiang, 1968). Thus, the source of some inhibitory sidebands in magnocellularis could be the result of inhibition in the afferent inputs, which is apparently not seen in cats. We must be careful to point out again, however, that because of the consistently high sensitivity of the bird auditory nerve fibers, uncontrolled noise sources (e.g., animal noises) may well contribute to what we think of as "spontaneous" activity.

The response histograms observed in both magnocellularis and angularis were quite similar to those seen in cat cochlear nuclei (Godfrey et al., 1975; Pfeiffer, 1966; Young and Brownell, 1976; Goldberg and Brownell, 1973). The same types of response patterns to best frequency tones were seen in nucleus angularis as have been reported for the DCN in unanesthetized cats, i.e., primary-like, pauser, and onset. Godfrey et al. (1975) have carefully classified response histograms of cells in both DCN and PVCN of barbiturate anesthetized cats and also find these types of patterns. Their "on-type I" cells respond "virtually only to the onsets of bursts" as do our onset units. The "on-type I" cells are found only in the PVCN. All of our onset cells were located in the medial third of nucleus angularis, which is said to be homologous with PVCN.

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