Organization and Development of Brain Stem Auditory Nuclei of the Chicken: Tonotopic Organization of N. magnocellularis and N. laminaris

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ABSTRACT Extracellular recordings of responses to tone-burst stimulation were used to determine the tonotopic organization of n. magnocellularis (NM) and n. laminaris (NL) in hatchling chickens. NM cells show "primary-like" response patterns to ipsilateral stimulation, and are arranged in dorso-ventral isofrequency columns. Units responding to the highest frequency tones (about 4,100 Hz) are situated at the rostromedial pole of the medial division. Units with lower characteristic frequencies (CF's) are found at successively caudal and lateral sites, until extremely low CF's (< 500 Hz) are represented dorsoventrally in the caudolateral tail of the lateral division. No evidence was found of auditory input to the region which receives projections from the macula lagena. NL receives polarized, binaural, excitatory input. Units have similar CF's and thresholds to tones presented to either ear. The tonotopic organization in NL matches that found in NM — high CF's rostromedially and low CF's caudal and lateral. Quantitative procedures were developed for relating CF to the position of a unit within either nucleus. These analyses account for 79% and 89% of the frequency variance found within NM and NL, respectively, and predict the CF of a neuron by its position within each nucleus.

Three lines of research have led to increasing interest in the avian auditory system. First, a large body of literature on bird vocalization (Hinde, '69) has led to studies relating behavioral capacities to structure and function of the auditory system (Schwartzkopff, '55; Winter, '63; Konishi, '69, '70). Second, the unique phylogenetic position of *Aves*, as the other major class evolving from reptiles, has led to valuable insights into the possible origins of the mammalian neural structures (Bock, '69; Boord, '69; Nauta and Karten, '70) and increased understanding of the avian auditory pathways (Boord, '61, '68; Boord and Rasmussen, '63; Karten, '67, '68). Finally, a body of research by Gottlieb ('71), indicating that species-typical vocalizations may influence the development of perceptual abilities, has provoked interest in the ontogeny of the avian auditory system (Konishi, '73; Saunders, Coles and Gates, '73).

This paper represents the first of a series of investigations on the organization and development of two brain stem auditory nuclei in the chicken: n. magnocellularis and n. laminaris. These nuclei were chosen for study because of their relatively simple relationships to the periphery and to each other, as well as their relatively homogeneous cytoarchitecture (Boord, '69). Nucleus magnocellularis receives its main afferent projections from cochlear and lagenar ganglion cells, central axons of which distribute in an orderly fashion to terminate in characteristic bulbs of Held on the cell bodies and dendrites of n. magnocellularis neurons (Boord and Rasmussen, '63). Nucleus laminaris neurons are arranged in a concave monocellular layer of cell bodies lying ventral, lateral, and rostral to n. magnocellularis. The binaural projection to n. laminaris from n. magnocellularis is spatially segregated such that the contralateral input terminates in the ventrolateral neuropil region while the ipsilateral axons terminate in the dorsomedial neuropil region (Ramón y Cajal, '08; Boord, '68; Parks and Rubel, '75). The cell bodies of n. laminaris appear to receive mixed ipsi- and contralateral innervation (Benes, Parks and Rubel, unpublished observations).

Neurophysiological investigations of n. magnocellularis in the pigeon (Stopp and Whitfield, '61) have stressed the similarities of mammalian and avian tuning curves and the relatively small frequency range that is characteristic of this and other avian species. Konishi ('69) concentrated on the relationship between the frequency-intensity thresholds of brain stem auditory units and species-typical vocalizations of songbirds. The general pattern of tonotopic organization of brain stem auditory units has been described for the house sparrow and embryonic duckling by Konishi ('69, '73). Yet a detailed description of tonototopic organization in n. magnocellularis is not available for any avian species.

Less data are available on n. laminaris. Although the spatially-segregated bilateral innervation pattern has been confirmed experimentally (Boord, '68), information on binaural response properties and tonotopic organization of the cells is not available.

In the present investigation standard microelectrode recording procedures were used to study the response of n. magnocellularis and n. laminaris units to tonal stimuli applied to one or both ears of hatchling chickens. The binaural nature of n. laminaris cells is described, as is the tonotopic organization within each nucleus. Quantitative procedures, relating characteristic frequency to neuronal position, were developed in order to assess the stability of tonotopic organization across subjects and to generate equations predicting the characteristic frequency of a neuron from its position in n. magnocellularis or n. laminaris.

MATERIALS AND METHODS

Subjects and surgical procedures

Subjects for the present study were 52 Red Cornish or White Leghorn hatchling chickens 5–15 days old. Eggs were obtained from a commercial breeder and incubated in our laboratory. Weights ranged from 50 to 150 gm.

Chicks were initially anesthetized with intraperitoneal injections of 0.003 ml/gm body wt. of Equithesin (Jensen-Salsbery Laboratories) and hydrated with 2–3 ml of 5% dextrose in 0.9% NaCl. The feathers were clipped from the head area including the ear-flaps. Following a midline incision, the skin overlying the posterior cranial bones was retracted and small woodscrews were inserted into the parietal bones over each hemisphere. Dental acrylic, attached to the beak and the skull screws, secured the bird's head to a specially designed head-holder, the neck muscles were retracted to expose the foramen magnum, and the occipital bones were removed to expose the cerebellar cortex. In most subjects, the middorsal sinus was ligated in two places between the anterior cerebral vein and the middle cerebral vein. The sinus was then cut and the entire cerebellum aspirated to expose the floor of the fourth ventricle. Cochlear nerve fibers coursing rostromedially over the medullary surface and a large blood vessel which lies above the caudomedial portion of n. magnocellularis served as landmarks. In later experiments the cerebellum and vascular system were left intact and the electrode was introduced through the cerebellum. This procedure results in less pulsation and, therefore, superior recording conditions.

Throughout the surgical preparation and the recording session, body temperature was maintained at 38–39°C. Nociceptive reflexes were monitored and controlled by intramuscular injections of Equithesin.

Stimulus presentation

Stimuli were white-noise or pure-tone bursts, 50 msec in duration, with 10 msec rise and fall times. The inter-stimulus intervals were two seconds in early experiments and one and one-half seconds in later experiments. White noise was used to locate units and tone bursts were used for CF determination. Sound presentation equipment included a Wavetek model 134 function generator, Grason-Stradler 901B noise generator and 829E electronic switch, and Hewlett-Packard 350D attenuator. Stimuli were delivered to either ear through brass adaptors which were attached to calibration earphones from the Brüel and Kjaer Probe Microphone Kit (B & K no. UA0040). The brass adaptors, with earphones attached, were sealed to the external auditory meatus to create a "closed system." Each earphone assembly was calibrated over the range from 0.1 to 10 kHz using a B & K 1/4" condenser microphone

(no. 4135) and a B & K Frequency Analyzer (no. 2107). Sound frequencies were determined on line, using a General Radio 1151A digital Time and Frequency Meter. Since earphone assemblies were attached to both ears, "characteristic frequency" (CF) and threshold (re 0.0002 dynes/cm^2) could routinely be determined for stimulation of each ear.

Recording procedures

Cellular activity was recorded through glass-insulated tungsten microelectrodes constructed by procedures based on Hubel ('57), Baldwin et al. ('65), Merrill and Ainsworth ('72), and Parker et al. ('73). Electrodes had 5–20 μm of exposed tip and a shaft diameter of 10–40 μm. Potentials from the electrode, with reference to a grounded neck muscle, were amplified, filtered to pass 300 Hz–10 kHz, displayed on one beam of a storage oscilloscope (CRO), aurally monitored, recorded on magnetic tape (Sony no. TC-650) and led to a pulse height discriminator (Frederick Haer). The output of the discriminator was used for compiling post-stimulus-time (PST) histograms (Ortec Model 4620/4621) and was continuously monitored along with the stimulus on a second beam of the CRO. A second CRO was used to monitor the waveform of neural unit activity and the output of the histogram analyzer. Evoked potentials were displayed by eliminating the high pass filter, and they were averaged using an Ortec Signal Averager.

Under visual inspection, through a Zeiss operating microscope, the electrode was lowered to the pial surface, taking care to avoid any blood vessels. Contact with the surface could be detected over the audio monitor and by a slight dimpling of the medulla. In preparations where the cerebellum was intact, contact with the medulla was indicated by a sharp rise in background activity and small units responding to auditory stimuli. The electrode was slowly lowered into the tissue while broad-band noise bursts (approximately 80 dB re 0.0002 dynes/cm^2) were presented to both ears. When units either activated or inhibited by the stimulus were encountered, the microdrive was stopped and the response was "tuned." Both well-isolated units and clusters with 2–4 individual spikes were used. Within the nuclear groups, individual units of a small cluster consistently showed similar response properties.

Characteristic frequency determination

The characteristic frequency (CF) was first determined manually by slowly changing frequency and intensity until the lowest threshold combination was determined. This procedure was carried out independently for stimulation of each ear. The manually-determined CF was then confirmed by compiling PST histograms to that frequency and to frequencies 100–200 Hz above and below that CF at 5–15 dB above the thresholds. Units were considered binaural if threshold intensities to stimulation of each ear was within 10 dB.

Each active neural unit or small cluster of units encountered within an electrode penetration was analyzed and the corresponding electrode depth was noted. Small "marking" lesions were placed at points of special interest (usually as the electrode was withdrawn) by passing 10–20 μamps for 5–10 seconds through the recording electrode (the anode). Penetrations were made in rows and the position of each penetration was marked on a grid. Penetrations were at 0.2 mm or 0.3 mm intervals within a row and rows were spaced 0.3 or 0.4 mm apart. The rows were usually started at the most rostromedial site in order to avoid damage to the majority of afferent fibers which course rostromedially. In most experiments only one or two rows of electrode penetrations were carried out in order to facilitate localization of electrode tracks.

Histological procedures

At the termination of each experiment the animal was sacrificed by an overdose of Equithesin and a knife cut was made through the brain stem at the angle of the electrode penetrations and parallel to the row(s). The head was then immersed in Bouin's solution for 24 hours, the brain stem was removed, blocked and embedded in paraffin. The block was serially sectioned at 10–20 μm in a plane parallel to the knife cut. All sections were mounted and stained with thionin.

The positions of the electrode tracks were first identified by viewing sections alternately with phase and transmitted

light microscopy. Reconstructions were then prepared by tracing electrode tracks onto × 256 projections of the appropriate sections. Tissue shrinkage was determined for each brain by one or more of the following measures: distance between two adjacent electrode tracks within a row; distance between two marking lesions placed within a single electrode track; or distance between the brain surface and a marking lesion. Although shrinkage varied considerably between animals (14–56%), the different measures yielded consistent results within any one brain. Only data obtained from recording sites within a nuclear boundary are reported, although auditory responses were reliably recorded from non-nuclear regions surrounding n. magnocellularis and n. laminaris.

Quantitative analysis

The "mapping" procedures outlined above are relatively standard and have proven reliable for qualitative descriptions of sensory and motor system organization within a large variety of animals (Rose et al., '59; Potter, '65; Johnson et al., '68; Allman and Kaas, '71; Rubel, '71). Less common are quantitative analyses of nuclear organization which are based on electrophysiologically-defined response properties of the neurons. Thus, the methods used for these analyses are described in more detail in figure 1 which diagrammatically shows the method used in the present study.

An accurate two-dimensional reconstruction of each nucleus was used to determine the medio-lateral and rostro-caudal percentile positions of recording sites for each experimental brain sectioned in the coronal or parasagittal plane. The data, relating characteristic frequency to rostro-caudal and medio-lateral position, were then subjected to individual and multiple linear regression analyses. These statistical analyses were used to indicate the extent to which the characteristic frequency of a neuron can be predicted by its position in n. magnocellularis or n. laminaris.

RESULTS

Nissl-stained sections through the brain stem at two rostro-caudal levels, showing the normal appearance of n. magnocellularis and n. laminaris in the hatchling chicken, are reproduced in figures 12 and 13.

The electrophysiological data reported here are representative of findings from 132 responsive electrode penetrations through n. magnocellularis and/or n. laminaris. These penetrations yielded 526 units or unit clusters, within the nuclear boundaries, for which the CF was determined. Although spike amplitude generally increased by 200–400 μv as an electrode entered the nuclear areas, clear unit responses were regularly found while the electrode traversed the region occupied by afferent axons above n. magnocellularis, and in the neuropil regions between n.

Fig. 1 Method for constructing planar projections of n. magnocellularis (NM) and n. laminaris (NL). Serial sections through the brain stem of a Red Cornish hatchling chicken were cut at 10 μm in the coronal plane and stained with thionin. Beginning with the most rostral section through n. laminaris (NL), the right dorsal medulla was traced at a linear magnification of × 128. A stage micrometer was used to insure that all areas of the field were equally magnified. One such tracing (section no. 45) is shown at the top of the figure, with n. magnocellularis (NM) and n. laminaris (NL) shaded and stippled, respectively.

The mediolateral extent of each nucleus was then determined (shaded and stippled bars) and indicated in the appropriate medio-lateral and rostro-caudal position on a horizontal plane, as shown for section no. 45 by the black bars in the middle part of the figure. The rostro-caudal dimension was, of course, also subjected to linear magnification of × 128. Thus, the middle part of the figure shows an accurate horizontal projection of n. magnocellularis (shaded) and n. laminaris (stippled) with reference to the midline of the brainstem (shown at left). The area outlined by the dashed lines, at the caudolateral pole of the nuclear complex is the cytoarchitecturally distinct ventrolateral subdivision (NM_{VL} of Boord, '69), where n. magnocellularis and n. laminaris merge. This area, although unresponsive to sound (RESULTS), is traditionally considered part of the avian cochlear nuclear complex (Boord and Rasmussen, '63) and is therefore included in our maps. It has been labeled "Lag," however, in view of its innervation from the lagenar, rather than cochlear, portion of the posterior ramus of the eighth nerve (DISCUSSION).

In the lower section, the planar projections of the two nuclei are separated, but retain their rostro-caudal and medio-lateral orientations. Each nucleus was divided into a percentile grid and the position, in percentile from posterior-to-anterior and lateral-to-medial, of recording sites, from any brain which was sectioned in the parasagittal or coronal plane, could be entered on this grid.

Lag, lagenar projection area; Na, n. angularis; VeL, nucleus vestibularis lateralis; VeM, n. vestibularis medialis; Ta, nucleus tangentialis (Cajal).

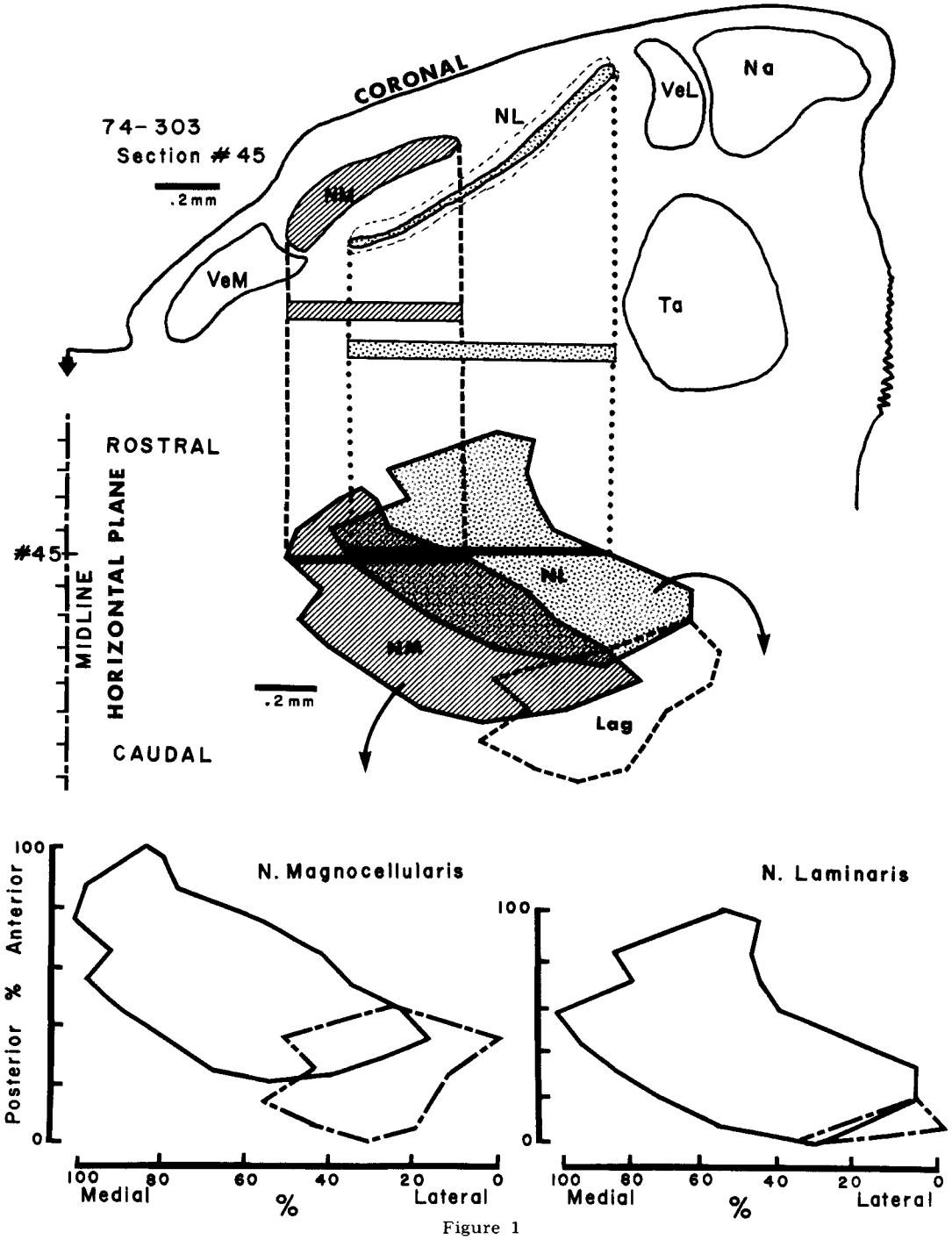

CORONAL
74-303
Section #45
.2mm
NM
NL
VeM
VeL
Na
Ta
ROSTRAL
#45
MIDLINE
HORIZONTAL PLANE
CAUDAL
.2mm
NL
NM
Lag
N. Magnocellularis
N. Laminaris
Posterior % Anterior
100
0
100 80 60 40 20 0
Medial
%
Lateral

Figure 1

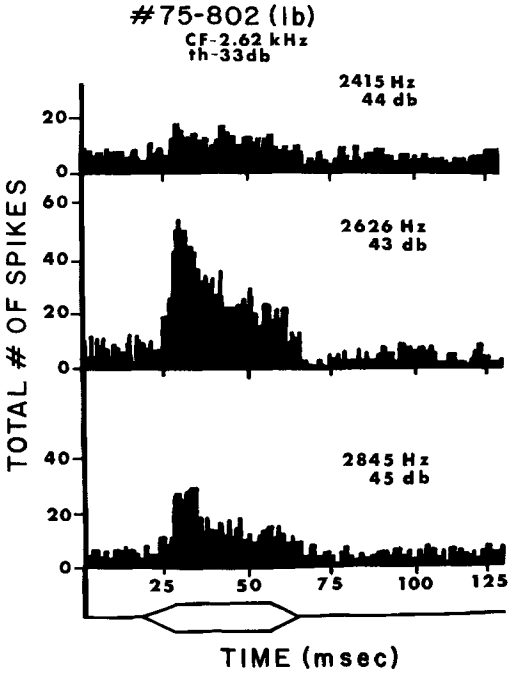


Fig. 2 Post-stimulus-time histograms from unit lb of experiment 75-802. Unit was in n. magnocellularis, CF was 2.62 kHz, threshold (th) to ipsilateral ear stimulation was 33 dB (SPL), threshold to contralateral ear stimulation was 58 dB (SPL). Histograms, summed over 128 repetitions, show "primary-type" response pattern and clear decrement in responding at about ±200 Hz from the CF. Bin width = 1.0 msec.

magnocellularis and n. laminaris as well as beneath n. laminaris (figs. 12, 13). Responses recorded from these fiber and neuropil regions can often be misleading with regard to tonotopic organization since axonal responses may be recorded far from the terminals or postsynaptic neurons. For example, eighth nerve fibers with high CF's pass above most of n. magnocellularis before turning ventrally to terminate in the rostro-medial pole of this nucleus. For these reasons, only recording sites for which histological analyses confirm a nuclear location of the electrode tip can be used for determining the tonotopic organization of these nuclei.

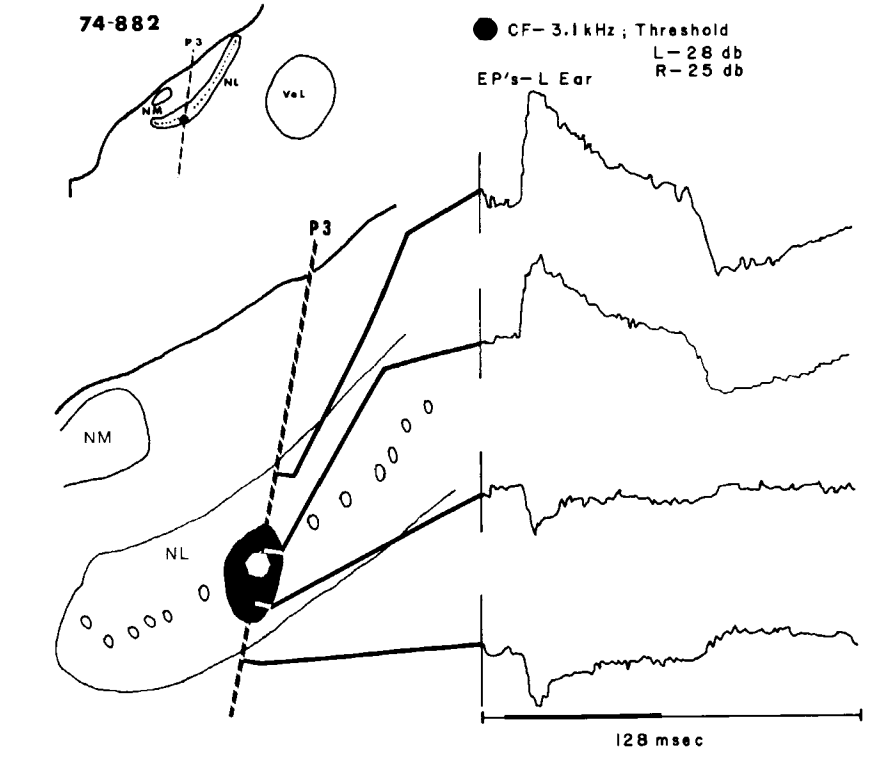
Response properties

Units in n. magnocellularis show "primary-type" response patterns similar to those found in auditory nerve fibers of mammals and birds (Kiang et al., '65; Sachs et al., '74) and the anteroventral cochlear nucleus (AVCN) of mammals (Kiang et al., '73). Following tone onset there is a rapid increase in firing rate followed by a decrease to a sustained level. The end of a tonal stimulus is marked by a sharp fall in firing rate below the spontaneous level and then a gradual return to the spontaneous firing rate. A typical set of PST histograms is shown in figure 2. Activation of units in n. magnocellularis via stimuli applied to the contralateral ear always required tones 15–30 dB more intense than ipsilateral stimuli. Since such differences are of the order expected from non-neural sound transmission (Sachs et al., '74) units in n. magnocellularis probably receive only ipsilateral auditory input. Although the complete response-areas were not systematically determined in this investigation, units often showed inhibitory frequency bands on one or both sides of the CF. Purely inhibitory units, as indicated by suppression of spontaneous activity, were not encountered.

The most conspicuous difference between response properties found in n. magnocellularis and n. laminaris is the binaural input to the latter nucleus. Figure 3A shows the change in averaged evoked potentials consistently found as an electrode passes through n. laminaris, while tonal stimuli are presented to the contralateral ear. At the position of polarity re-

Fig. 3 Results from an electrode penetration through n. laminaris. A. *Top left:* insert showing location of electrode track through the right n. laminaris. *Below left:* enlarged diagram of electrode track showing positions at which evoked potential averages were taken, and the position at which a unit response (white hexagon) was recorded. A marking lesion (blackened area) was placed at the position where the polarity reversal of the evoked potential occurred. *Top right:* CF and thresholds (SPL) for stimulation of each ear sufficient to activate the cellular response recorded at the position of the octagon. *Below right:* averaged evoked responses to tone burst stimulation of the contralateral (left) ear at the CF. Duration of tone burst is shown by darkened area along base line. Total trace duration = 128 msec, positive-going traces are up. Note polarity reversal of the averaged evoked response as the electrode passed through the cellular and ventral neuropil layers of n. laminaris.

B. Photomicrograph of section through certer of the marking lesion (pointer), indicating position at which averaged evoked potential, shown above, exhibited polarity reversal. Bar indicates 0.2 mm.


A
74-882
P3
NL
NM
VeL
CF– 3.1 kHz ; Threshold
L – 28 db
R – 25 db
EP's – L Ear
P3
NM
NL
128 msec

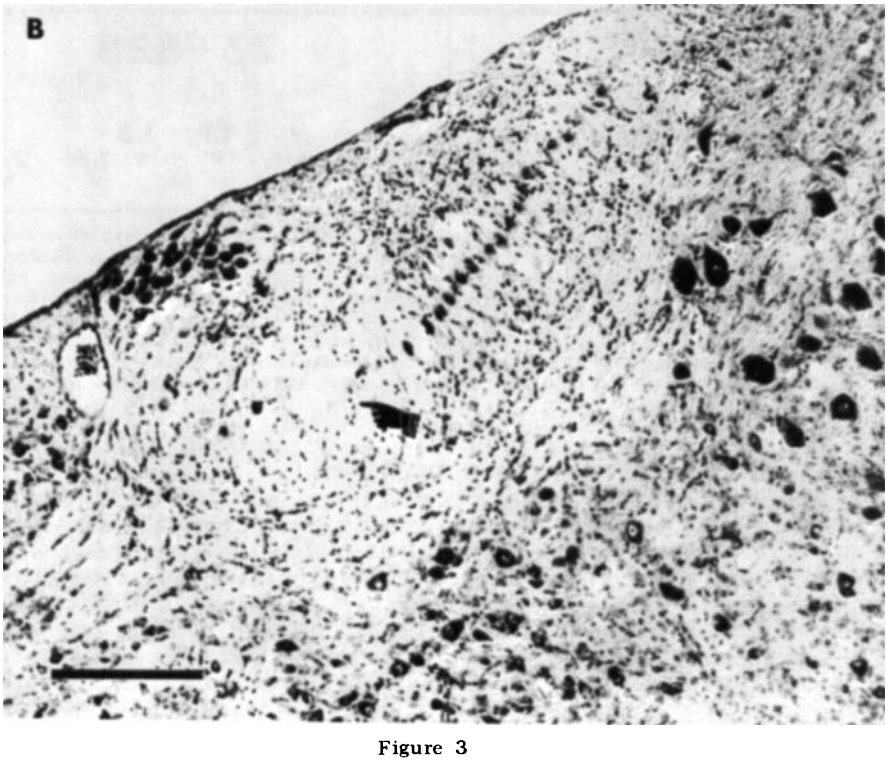

B

Figure 3

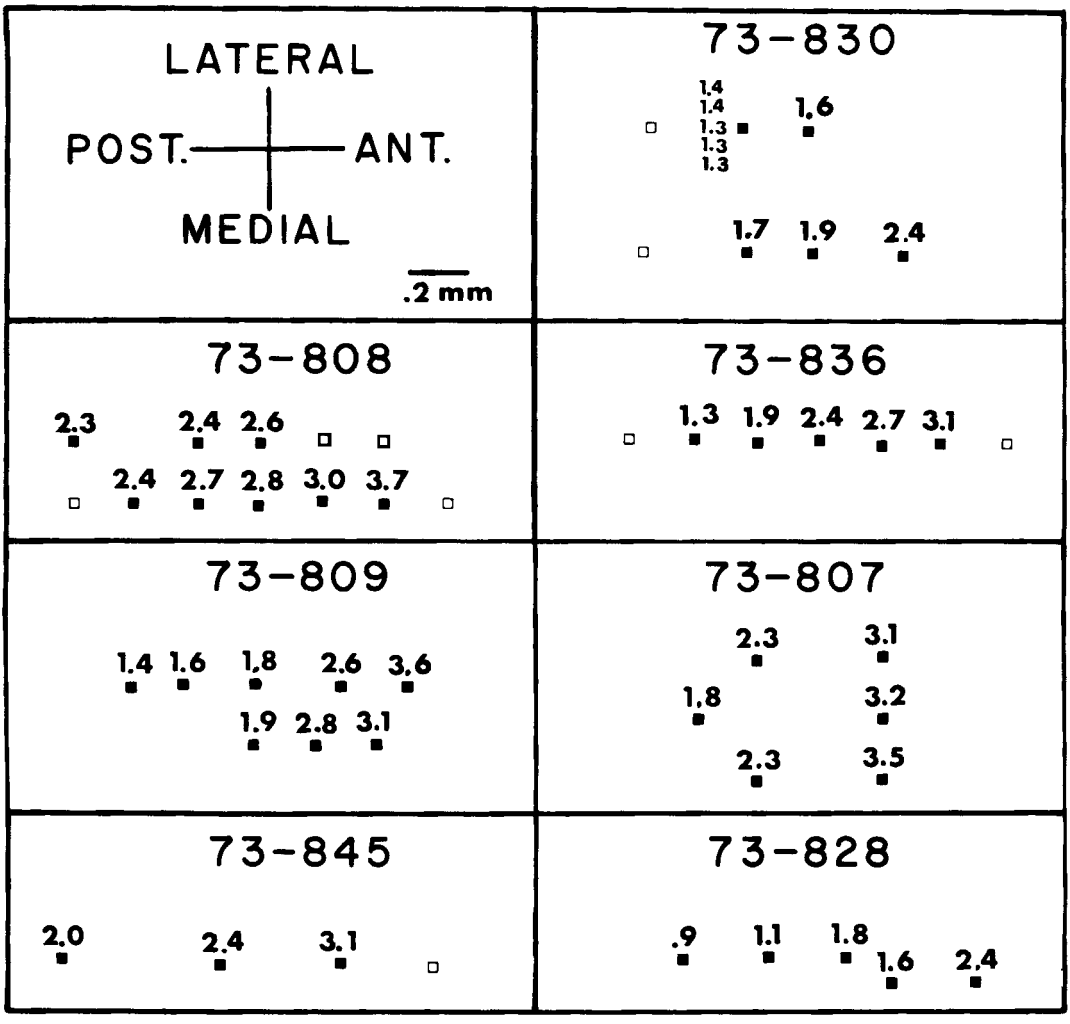


Fig. 4 Surface maps from seven representative experiments showing pattern of tonotopic organization found when the electrode tip was within n. magnocellularis and/or n. laminaris. Open squares indicate penetrations in which no responsive units were encountered. Filled squares indicate positions at which responsive units were encountered. For one penetration in experiment 73-830 the CF (kHz) of each unit encountered within the penetration is indicated. The CF's remained stable (within 0.5 kHz) throughout this and other penetrations. The median CF of the units found within each of the other penetrations is shown. Note that as the electrode is moved rostrally or medially, units with higher CF's are generally found.

versal a marking lesion was made while the electrode was withdrawn. The lesion is shown in figure 3B and its center (blackened area in fig. 3A) corresponds to the ventral side of the cellular lamina, suggesting that the potential originates from terminals innervating the ventral dendrites of n. laminaris cells. The cellular response recorded in this penetration (at the site of the white hexagon) had the same CF and similar thresholds for stimuli applied to either ear.

Units in n. laminaris appeared quite homogeneous in these properties, although a few ($< 10\%$) responded solely to either ipsilateral or contralateral stimulation (defined by a threshold difference of greater than 15 dB). All binaural units appeared to be of the excitatory-excitatory type in that there was no evidence of inhibitory

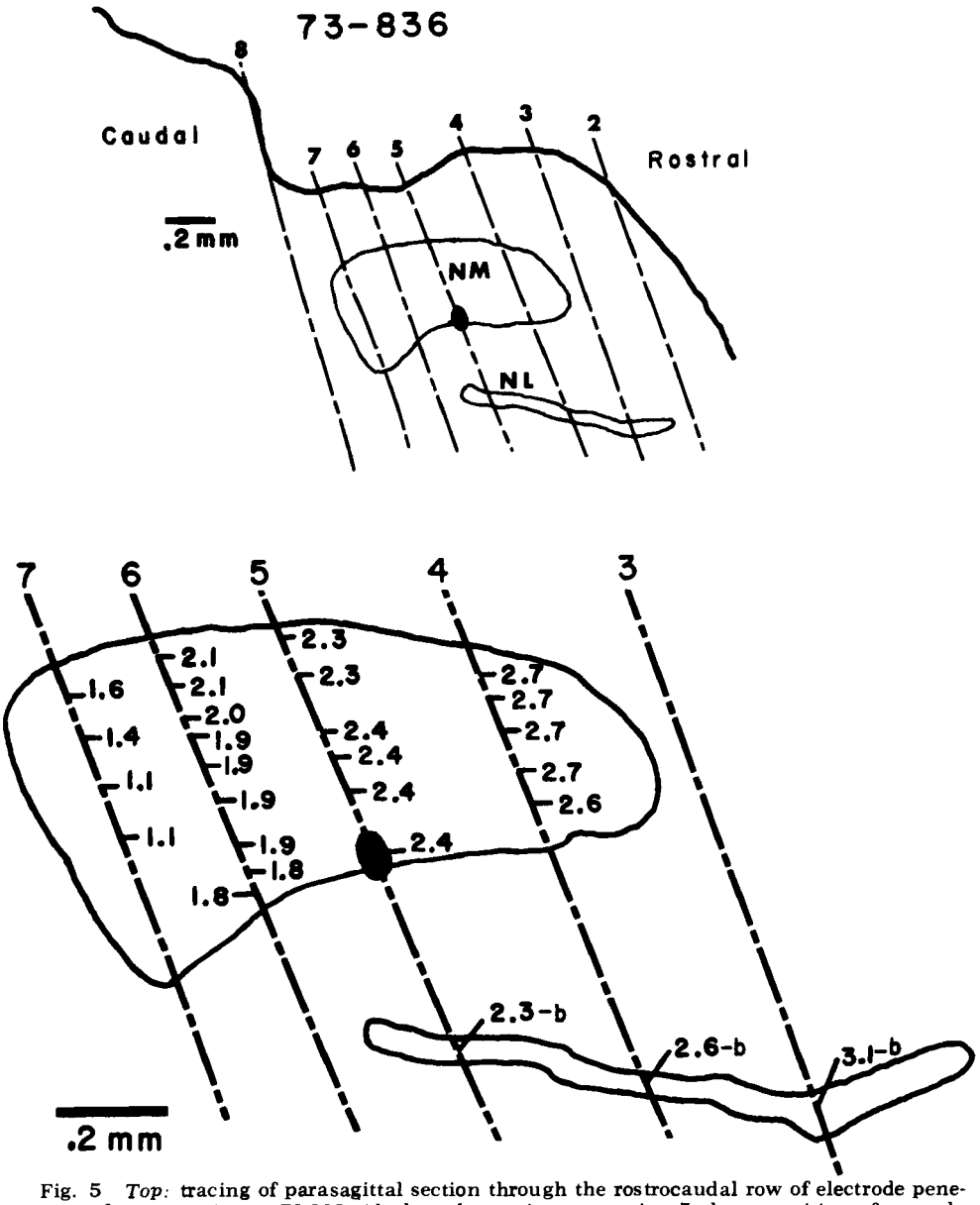


Fig. 5 *Top:* tracing of parasagittal section through the rostrocaudal row of electrode penetrations from experiment 73-836. Blackened area in penetration 5 shows position of a marking lesion. *Below:* enlargement of tracings of n. magnocellularis (NM) and n. laminaris (NL) showing positions and CF's (kHz) of units found in penetrations 3–7. Note columnar organization within NM and increasing CF's from caudal to rostral, found within each nucleus. Binaural units with the same CF for stimulation of either ear are indicated by "b."

interactions between stimuli applied to the two ears.

Tonotopic organization

Due to the columnar arrangement of n. magnocellularis neurons (see below) and the similarity of organization found in the two nuclei, a single electrode penetration usually encountered several units with similar CF's throughout its dorso-ventral extent. Thus, the general properties of tonotopic organization are best demon-

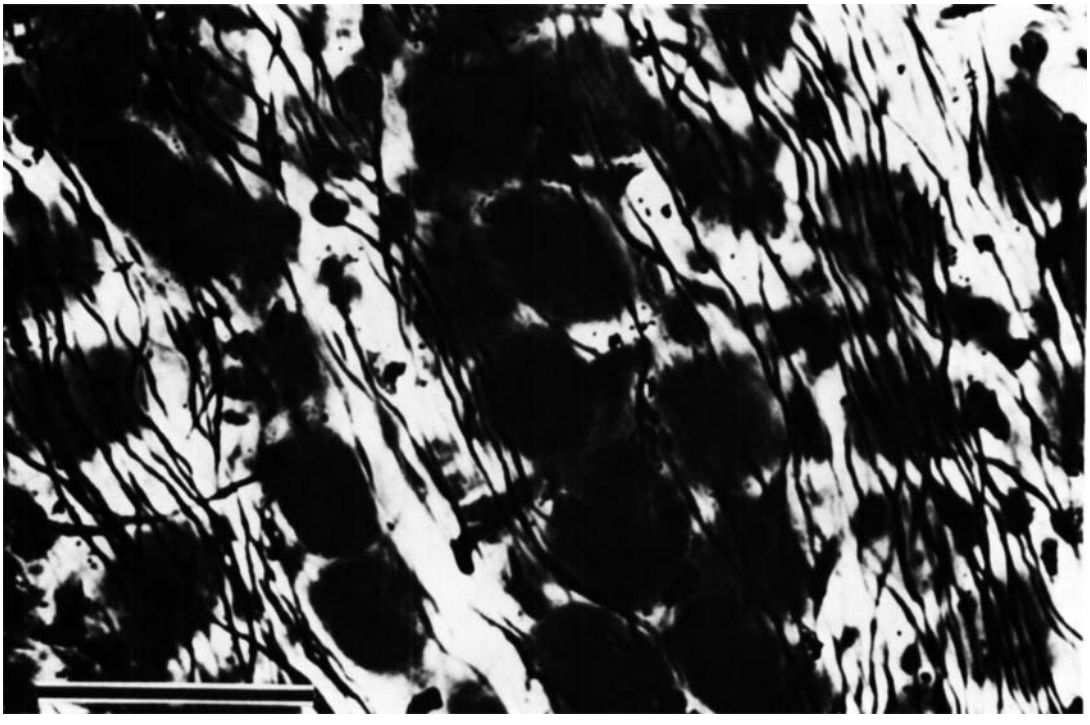


Fig. 6 Photomicrograph of a silver-stained coronal section through n. magnocellularis showing the morphological columnar organization of NM cells with afferent and/or efferent axons streaming down the columns. Dorsal at top, lateral at left. Bar indicates 50 μm. Modified Bielschowsky stain.

strated by a series of penetrations within a single preparation. The results from seven subjects, which are representative of what was found in all animals, are summarized in figure 4. A typical series of CFs found within the nuclei is shown for one penetration in animal no. 73-830, while the median CF in each of the other penetrations is indicated. The range of CF's within any of these penetrations was less than 350 Hz, although in several cases shown the electrode passed through both n. magnocellularis and n. laminaris. These data indicate a reliable tonotopic organization in the nuclei with progressively higher frequencies activating units located at successively rostromedial points in the nuclear complex.

The basic pattern of tonotopic organization found in these two nuclei is demonstrated in more detail by figures 5–9, which serve to exemplify the following points:

1. Columnar organization of n. magnocellularis. Throughout most of n. magnocellularis an electrode penetration encounters units with similar characteristic frequencies throughout the dorsal-ventral extent of the nucleus (figs. 5, 8). The only exceptions are found in the caudolateral portion of n. magnocellularis (pars lateralis of Boord and Rasmussen, '63), where units with low CF's are found and in the ventrolateral subdivision, which is unresponsive to auditory stimuli (fig. 7 and DISCUSSION).

The physiologically observed isofrequency columns in n. magnocellularis have anatomical correlates; the columnar arrangements of neuronal perikarya and the dorsoventral orientation of large fibers which appear to course between the cellular columns (fig. 6).

2. In n. magnocellularis, units with low CF's (under 600 Hz) are found in the most caudolateral position and are tonotopically organized with successively lower CF's represented at progressively ventrolateral positions (fig. 7).

3. Throughout the remainder of n.

magnocellularis a tonotopic organization is evident with sequentially higher CF's found rostral and medial to units displaying lower CF's (figs. 5, 8).

4. Units in n. laminaris are also arranged tonotopically in a manner which matches the organization found in n. magnocellularis; progressively higher CF's are found as the electrode is moved rostrally and/or medially in the nucleus (figs. 5, 8, 9).

In n. magnocellularis the range of unit CF's found in our sample was 200–4,100 Hz and in n. laminaris the unit CF's ranged from 170 to 3,800 Hz. It is expected that units with lower CF's are present in both nuclei but limitations of the sound delivery system prevented extensive sampling at very low frequencies.

Quantitative analyses

For the quantitative analysis of tonotopic organization in n. magnocellularis and n. laminaris each nucleus is considered only in two dimensions, rostro-caudal and medio-lateral. This can be justified by the facts that: (a) n. magnocellularis can be considered two-dimensional with respect to characteristic frequencies, due to the physiological demonstration of dorso-ventral isofrequency columns; and (b) n. laminaris in the hatchling chicken is morphologically a two-dimensional monocellular lamina (fig. 12).

With the methods described above and the planar projections (fig. 1) it is possible to specify the rostro-caudal and medio-lateral location of any responsive unit from any brain that has been sectioned in the

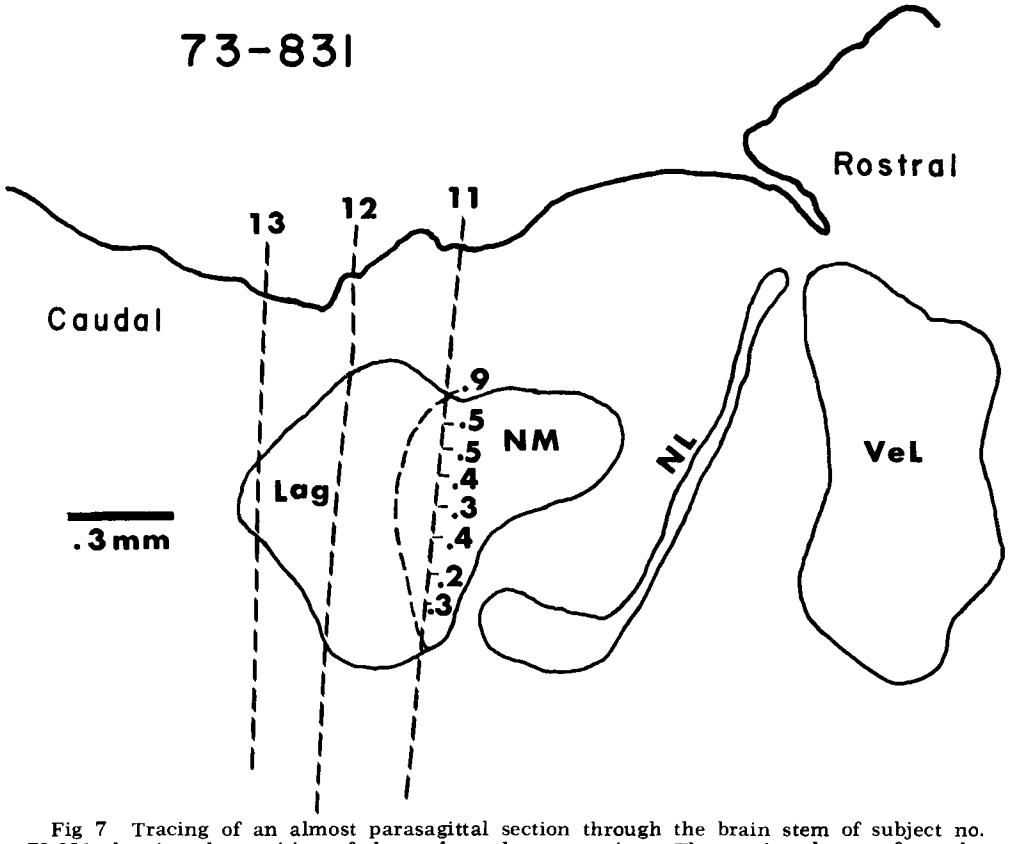


Fig 7 Tracing of an almost parasagittal section through the brain stem of subject no. 73-831 showing the position of three electrode penetrations. The section departs from the parasagittal plane such that the rostral portion is approximately 200 μm medial of the caudal part. Note the decreasing CF's from dorsal to ventral through the caudolateral portion of the lateral division of n. magnocellularis (NM). Lag indicates the projection area of the macula lagena. No units responsive to auditory stimulation were found as the electrode penetrated this nuclear region (penetrations 12, 13). NL, n. laminaris; VeL, n. vestibularis lateralis.

coronal or parasagittal plane. Thus, the posterior-anterior and lateral-medial percentile locations serve as coordinates to specify the location of a unit on a Cartesian surface, and the relationships between the characteristic frequencies and anatomical locations can be quantitatively analyzed. This analysis has been completed for the

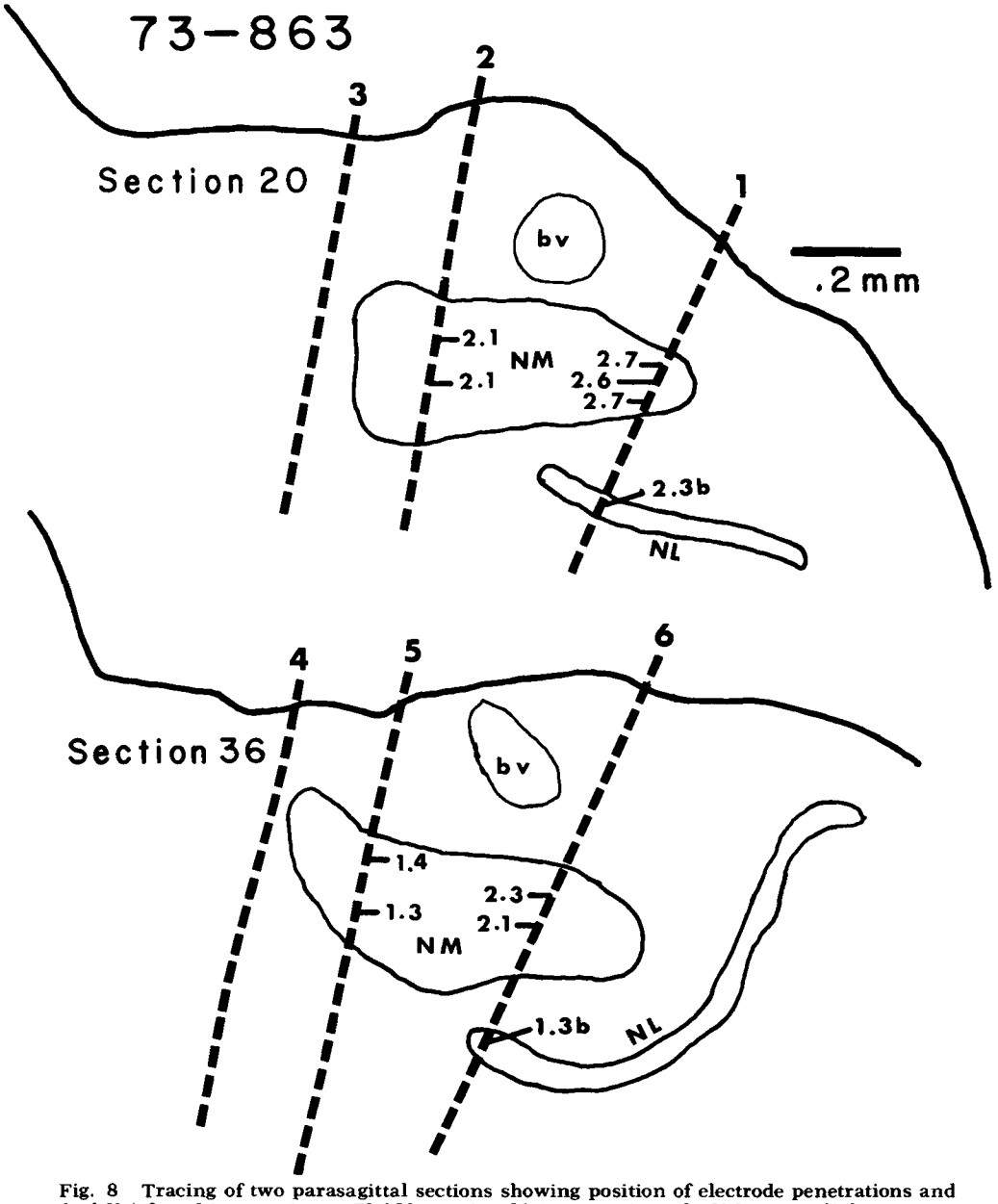


Fig. 8 Tracing of two parasagittal sections showing position of electrode penetrations and CF's (kHz) found in experiment 73-863. Section 20 is approximately 350 μm medial to section 36. The rostral direction is to the right. Note that within n. magnocellularis (NM) as the electrode is moved rostrally units with higher CF's are found, and that within both NM and n. laminaris (NL) medial penetrations produce units with higher CF's than lateral penetrations (compare penetrations 2 and 5; 1 and 6) bv, blood vessel.

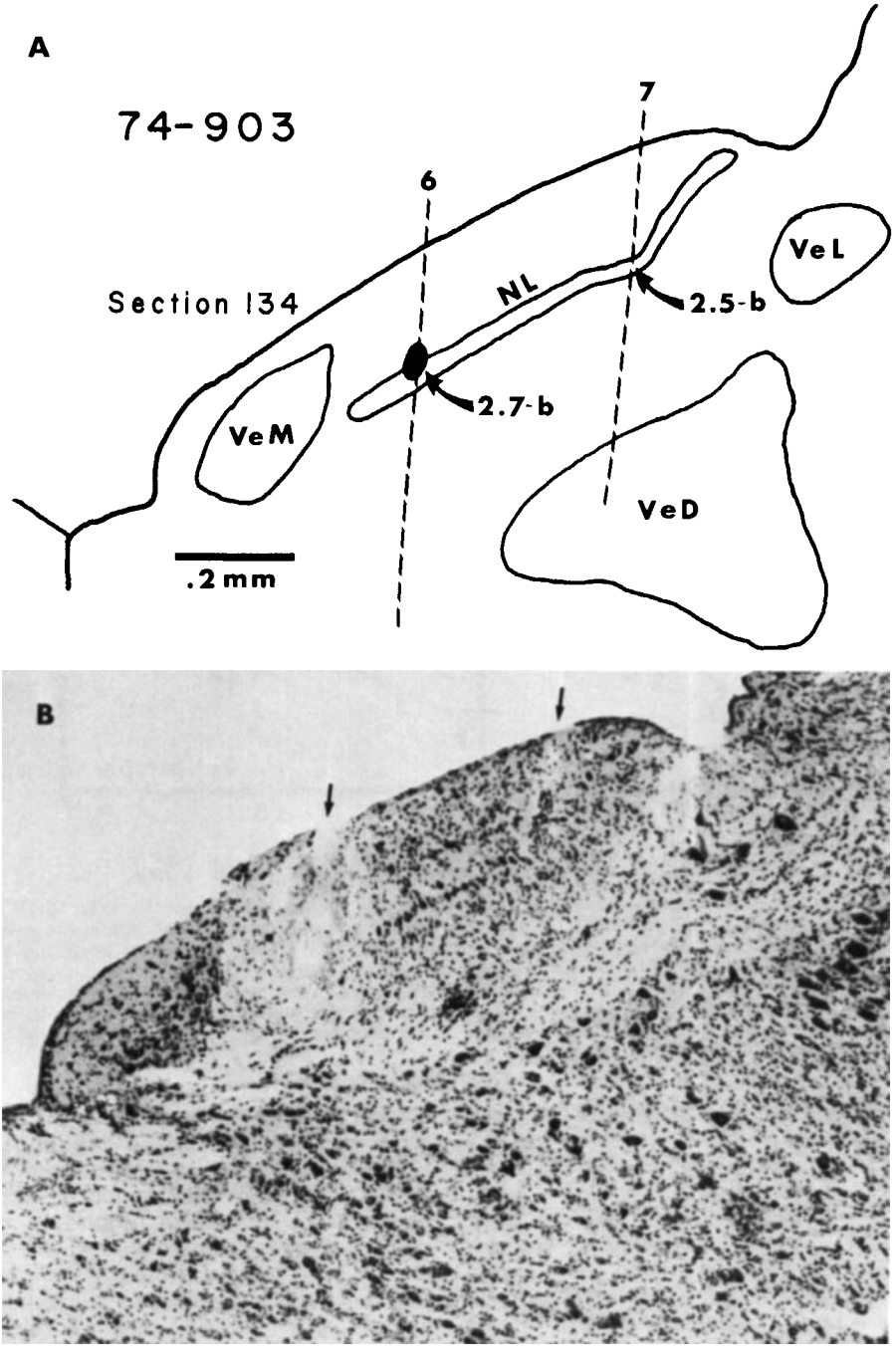


Fig. 9 A. Tracing of coronal section showing position of two electrode penetrations and CF of binaural units found as the electrode penetrated n. laminaris (NL). Note that a higher CF was found in the more medial penetration (no. 6). Blackened area shows position of marking lesion. VeD, n. vestibularis dorsalis; VeL, n. vestibularis lateralis; VeM, n. vestibularis medialis. B. Photomicrograph showing position of electrode penetrations (arrows).

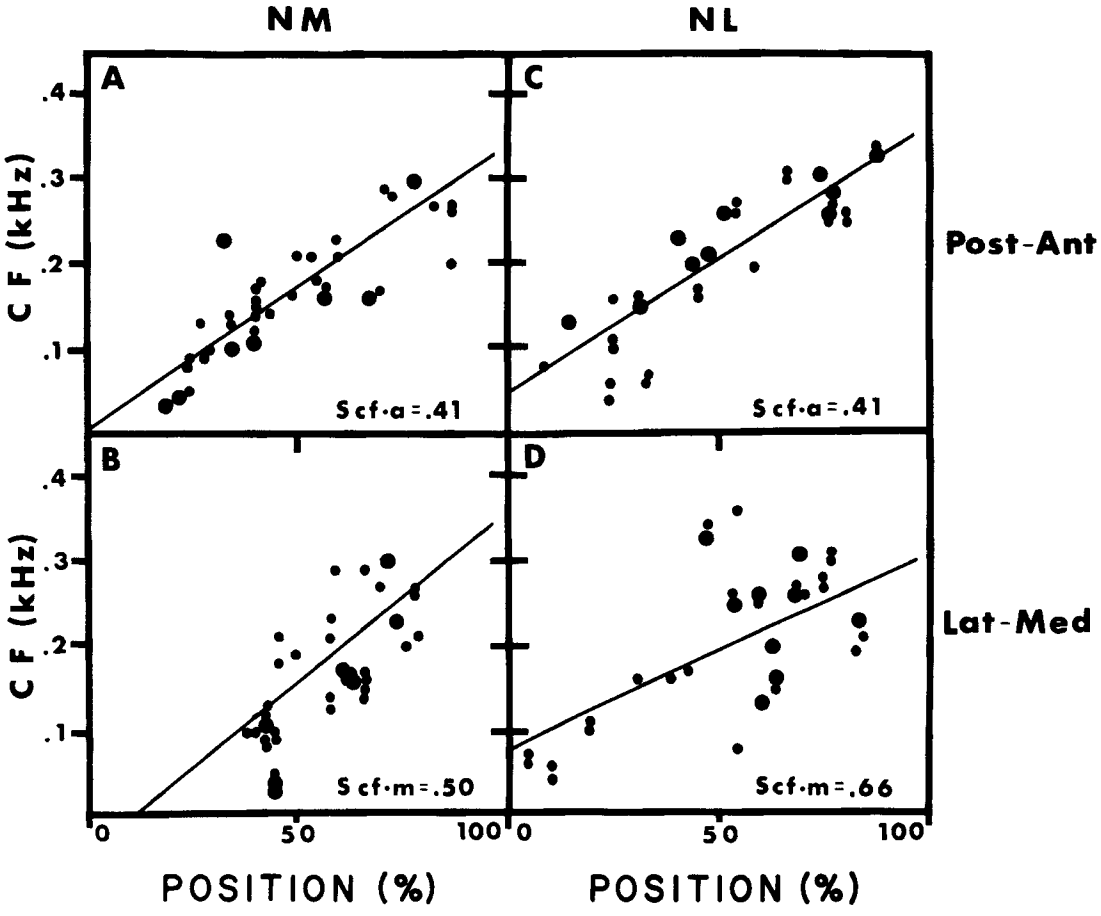


Fig 10 Scatter plots, linear regressions, and standard errors of estimate in kHz (Scf), relating characteristic frequency (CF) of units to posterior-anterior and lateral-medial percentile position of electrode tip in n. magnocellularis (NM) and n. laminaris (NL). Large dots show positions at which more than one unit having the same CF was found. A. Regression of CF on posterior-to-anterior percentile position in NM (48 units). B. Regression of CF on lateral-to-medial percentile position in NM (48 units). C. Regression of CF on posterior-to-anterior percentile position of units in NL (45 units). D. Regression of CF on lateral-to-medial percentile position of units in NL (45 units). a, anterior position; m, medial position.

data derived from 14 subjects which yielded 48 units or distinct clusters in n. magnocellularis and 46 units or clusters from n. laminaris.

The linear regressions of CF on each positional dimension (posterior-anterior and lateral-medial) are shown for n. magnocellularis and n. laminaris in figure 10. There is a highly significant (p's < 0.001) relationship between both positional dimensions and CF in both nuclei. In n. magnocellularis, CF correlates with posterior-anterior position ($r = 0.84$) and lateral-medial position ($r = 0.75$). In n. laminaris correlations of 0.87 between CF and posterior-anterior position and 0.60 between CF and lateral-medial position were found. These data indicate that across animals, as well as within each individual animal, there is a very uniform tonotopic organization within each nucleus. Furthermore, this tonotopic organization follows the long axis of each nucleus seen on the planar projections in figure 1; from caudolateral to rostromedial. The apparent lack of sampling in the lateral 40% of n. magnocellularis is primarily because this area is predominantly associated with the lagenar

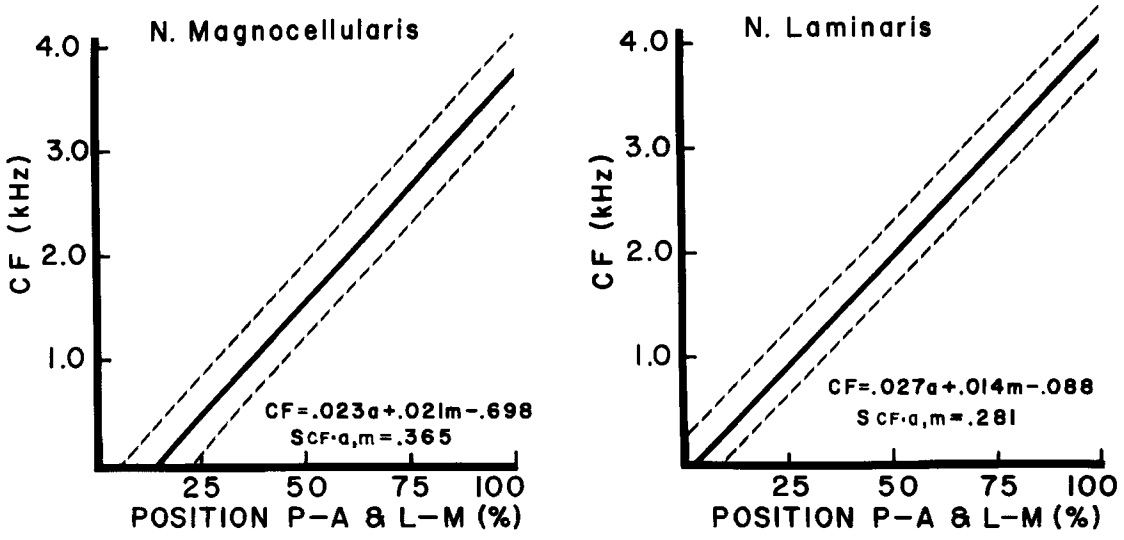


Fig. 11 Multiple linear regressions (± 1 standard error of estimate) relating CF of units to posterior-to-anterior and medial-to-lateral percentile position of the electrode tip in n. magnocellularis or n. laminaris. Regression equation and standard error of estimate in kHz (SCF) are shown below the regression lines.

projection area (fig. 1). Since units in this area were unresponsive to sound, they were not included in these analyses.

Since both of the positional variables relate to characteristic frequency and represent orthogonal dimensions, a multiple linear regression analysis was completed for each nucleus. The multiple regression lines ± 1 standard error, the regression equation, and the standard error of estimate are shown in figure 11 for each nucleus. These analyses indicate that the CF of a unit in either n. magnocellularis or n. laminaris can be accurately predicted by its position within the nucleus. The regression function accounts for 78% of the variance in frequencies found in n. magnocellularis (r = 0.88), and 89% of the frequency variance observed in n. laminaris (r = 0.94).

DISCUSSION

The chief contribution of the present study is the quantitative description of tonotopic organization within n. magnocellularis and n. laminaris. Quantitative procedures, based on distance from the suprasylvian sulcus, have been used in previous studies to analyze tonotopic organization of the cat auditory cortex (Hind et al., '60; Evans et al., '65). The aim of these investigations was to determine *if* neurons are arranged in a consistent tonotopic manner, and the results have been interpreted in different ways (Whitfield, '67). In n. magnocellularis and n. laminaris of the chicken the existence of tonotopic organization was not questioned. Rather, the aims of the regression analyses in the present study were to determine the amount of the variability in unit characteristic frequencies accounted for by knowledge of the position of the unit within each nucleus, and the accuracy with which a neuron's CF can be predicted by knowledge of its position in each nucleus.

The multiple linear regressions were effective in that they accounted for 78 and 89% of the frequency variance in n. magnocellularis and n. laminaris, respectively. However, there are several sources of error which make it probable that tonotopic organization in both nuclei is much more precise than is evident from the standard errors plotted in figure 11. Among the major sources of within-subject error are: (1) errors in determination of characteristic frequency which, in some cases, could be as much as 150 Hz; (2) it is possible that in some cases recordings were made from fibers of passage; (3) although only brains sectioned in the coronal or parasagittal planes were used for the quantitative analysis, any variability in sectioning angle

introduces error in the percentile estimates; and (4) any deviations of the tonotopic organization from the horizontal plane, as occurs in the caudo-lateral end of n. magnocellularis and the rostral end of n. laminaris, introduces error into the analysis. Between-subject error is contributed by the combination of data from many subjects in preparing the composite maps. Although this is necessary to determine regression functions for use in future investigations (see below), small differences in nuclear size and shape, differences in sectioning angle and slight differences in histological procedures introduce variability into the data. Although these factors were not controlled in the present study the "close fit" of the multiple regression analyses indicate that they do not represent major sources of variation.

There are also reasons to assume that a non-linear function would be more appropriate for the analysis of sensory organization. Differential development of particular regions within sensory systems has been the subject of much investigation (e.g. Welker et al., '64; Konishi, '69). When the result is an increase in the relative cellular area devoted to a particular range of the energy spectrum or amount of receptor surface, a non-linear analysis may become progressively more appropriate. If, however, the hypertrophy is manifest in increased cellular density or in nuclear expansion in a dimension not related to the parameter under investigation (e.g. the dorso-ventral dimension of n. magnocellularis) linearity is not disrupted. In addition, Clopton et al. ('74) note that at some levels of the mammalian auditory system, particularly the basilar membrane and ventral cochlear nucleus, logarithmic functions relating distance to CF provide better predictions than linear functions. As the audible frequency range of animals increases, the difference betweem log and linear functions becomes increasingly important. Thus in avian species, which are characterized by a relatively small frequency range, a linear analysis is probably appropriate. More empirically, a logarithmic transformation applied to the frequency parameter of our data did not appreciably change the outcomes of the analyses presented above.

Despite errors introduced by use of composite data and linear analyses, the regression procedures have shown that the tonotopic organization of n. magnocellularis and n. laminaris is quite uniform across animals of approximately the same age and breed; and that a large proportion of the CF variation is related to the position of a neuron within each nucleus. In other words, it is possible to predict the CF of a neuron by its relative position within either nucleus.

The use of quantitative analyses of grouped data provides several advantages over traditional "mapping" methods. First, quantitative data of this nature provide an expandable data-base to which new observations can be added and with which the observations of other investigators can be directly compared (Clopton et al., '74). From such findings the replicability of our observations can be directly determined and variations among taxonomic groups can be quantitatively assessed.

A second advantage involves the discovery of other properties of neural organization besides receptotopic organization. For example, van Noort's ('69) observations on the AVCN of the cat, as well as the data presented above for n. magnocellularis, suggest that these nuclei are composed of isofrequency planes arranged in a tonotopic manner. While it is possible that all of the cells within a plane are functionally redundant, it is also conceivable that cells within one plane are systematically organized in some fashion. Quantification of the tonotopic dimensions makes it possible to systematically investigate orthogonal dimensions of the nuclei. In this respect two interesting questions are: Is the superior-to-inferior dimension of the basilar membrane represented orthogonally to the tonotopic organization in n. magnocellularis? Is the other dimension of the n. laminaris cell lamina systematically related to the position of a sound in space (i.e. to time differences of sound reaching the two ears; see Parks and Rubel, '75).

Quantification of sensory system organization across subjects also has important advantages for studying changes in neuronal properties as a function of age, experimental manipulations, or pathology. For example, independent prediction of

the neural elements normally activated by a particular environmental event (e.g. a particular tonal frequency band) makes it possible to study the specific effects of environmental enrichment or deprivation on the physiology, biochemistry and structure of those neural elements, as compared to cells normally activated by a different frequency range.

Comparisons with other avian species

The tonotopic organizatiom of n. magnocellularis observed in the present study is consistent with the results of previous investigations on other avian species. Boord and Rasmussen ('63) demonstrated that fibers innervating the distal portion of the pigeon basilar membrane terminate in the caudolateral region of n. magnocellularis and that successively proximal areas project to progressively rostromedial areas of n. magnocellularis. It is generally accepted that low-frequency sound is transduced in the distal portions of the basilar membrane and higher frequencies more proximally (Pearson, '72; Bekesy, '60). The results of Boord and Rasmussen, therefore, would predict the tonotopic organization observed in the present study and in previous studies on sparrows and embryonic ducks (Konishi, '69, '73).

The response characteristics of n. magnocellularis cells in the hatchling chicken also appear similar, with respect to laterality, post-stimulus-histogram contour, frequency range and intensity thresholds, to what has been reported previously. Characteristic frequencies of units observed in this study ranged from 0.2 to 4.1 kHz. Although a considerably larger range has been observed in many songbirds (Konishi, '69, '70) and Sachs et al. ('74) report a few pigeon auditory nerve fibers responding in the 5–6 kHz range, behavioral, evoked-potential and single-unit investigations of species without highly specialized vocal abilities show results similar to those of the present study (Heise, '53; Schwartzkopff, '68; Harrison and Furumoto, '71; Konishi, '73; Dooling and Saunders, '75; Saunders et al., '74). Thus a frequency range extending from less than 100 Hz up to approximately 4–5 kHz probably represents a relatively unspecialized avian form. One discrepancy between response characteristics of cells in n. magnocellularis of the pigeon (Stopp and Whitfield, '61) and those of the hatchling chicken is apparent. About one-fifth of the pigeon neurons could not be excited by pure-tone stimuli and had well-defined, purely inhibitory tuning curves. We observed many units in which the spontaneous activity could be inhibited by tonal stimuli, but no purely inhibitory units. In all cases the inhibitory frequencies were on one or both sides of an excitatory band. Whether this difference is due to age (adult vs 5–15-day-old), species (pigeons vs. chickens), or anesthetic (urethane vs. Equithesin) cannot be determined.

The tonotopic organization of n. laminaris has not been previously reported. It is noteworthy that this nucleus, which is innervated primarily or entirely by n. magnocellularis (Ramón y Cajal, '08; Sanders, '29; Boord, '68; Parks and Rubel, '75) is remarkably similar to m. magnocellularis in both shape and tonotopic organization. The organization of connections responsible for these similarities is the subject of the following paper (Parks and Rubel, '75).

Comparisons with other amniotes

Manley ('70b) did not find a clear indication of tonotopic organization in the cochlear nuclei of some chelonid, iguanid and gekkonid species, but n. magnocellularis of *Caiman* is tonotopically organized in a manner similar to that in birds (Manley, '70a). Although these interspecific differences in tonotopic organization were attributed to variations in the elongation and tapering of the basilar membrane (Manley, '70b, '72), the absence of detailed histological analyses of recording sites makes it possible that an existing tonotopic organization was obscured by the relatively small size of the cochlear nuclei in some species. In any case, all species of birds and reptiles for which a tonotopic organization of n. magnocellularis has been demonstrated present a quite similar pattern, suggesting that it may represent the basic plan of organization among non-mammalian terrestrial vertebrates.

Previous authors have suggested that the magnocellular nucleus of birds and reptiles is homologous to the anteroventral cochlear nucleus (AVCN) of mammals

(e.g. Ramón y Cajal, '08; Boord, '69). Neurons comprising the feline AVCN have been classified by several authors on the basis of both Nissl- and Golgi-stained material (e.g. Ramón y Cajal, '09; Lorente de Nó, '33; Osen, '69a,b; Brawer et al., '74). It is not possible to comprehensively relate the classification schemes of all authors (cf. Brawer et al., '74), however, some interesting similarities exist between the AVCN cytoarchitecture described by Brawer et al. ('74) and our observations on n. magnocellularis in the chicken and other avian forms. On the basis of Nissl-stained sections Brawer et al. ('74) divide the AVCN into anterior and posterior divisions which appear to correspond to the spherical cell areas, and globular and multipolar areas, respectively, of Osen ('69b). Nissl-stained sections through the medial and lateral divisions of NM of hatchling and embryonic chickens, embryonic ducks, and embryonic bobwhite quail reveal cellular characteristics similar to those described by the above authors; large and medium-sized spheroid and ovoid cells with no discernible dendritic processes and often containing an eccentric nucleus. Cells conforming to Osen's ('69b) "globular" type are interspersed throughout n. magnocellularis, but we have not seen any multipolar cells in the medial or lateral divisions. Observations of n. magnocellularis cells stained by Golgi-Kopsch and Golgi-Cox methods (Rubel and Parks, unpublished observations) indicate a preponderance of cells similar to the "adendritic bushy" and "bushy" types described by Brawer et al. ('74) plus a very few stellate neurons scattered throughout the nucleus. As in the pigeon (Boord and Rasmussen, '63), the only consistent difference between cells in the medial and lateral divisions of the nucleus of chicken, quail, and duckling is that in the lateral division cells tend to be slightly smaller.

In addition to the morphological similarities, AVCN and n. magnocellularis also share several physiological properties. Pfeiffer ('66a), Kiang et al. ('73) and Goldberg and Brownell ('73) report that units in the spherical cell region (anterior division of Brawer et al., '74) of the AVCN have narrow, excitatory tuning curves and display "primary-like" PST histogram patterns to tone burst stimulation. In n. magnocellularis, cells also have narrow tuning curves (Stopp and Whitfield, '61) and primary-type histogram patterns. The one contradictory finding, a large number of purely inhibitory units (Stopp and Whitfield, '61), was not confirmed in the present investigation. The characteristic triphasic waveform seen within AVCN of the cat (Pfeiffer, '66b; Goldberg and Brownell, '73), which is probably related to calycoid endings (Li and Guinan, '71), has not been investigated in the avian nucleus. Thus, several lines of converging evidence suggzst that the AVCN has remained in a relatively unchanged form during mammalian evolution, with the principal adaptation being that the embryonic cells, originating at the rhombic lip, migrate ventrolaterally in mammals and ventromedially in birds.

To our knowledge the only previous electrophysiological observations on n. laminairs are those of Erulkar ('55), Schwartzkopff ('63) and Stopp and Whitfield ('61). On the basis of evoked potential latencies Erulkar ('55) suggested that n. laminaris receives a direct projection from cochlear nerve fibers, and Stopp and Whitfield ('61), apparently, did not notice the binaural nature of these cells. On the basis of experimental anatomical studies (Boord and Rasmussen, '63), it is now generally accepted that the principal auditory input to n. laminaris is from the ipsilateral and contralateral magnocellular nuclei (Boord, '69), and Schwartzkopff ('68) indicated that the polarization of inputs to the two sides of n. laminaris neurons "shows up in electrophysiological experiments. . ." This nucleus, which is seen in some reptilian and apparently all avian species (Ariëns-Kappers et al., '36; Miller, '75; Rubel, unpublished observations) has been considered homologous to the medial superior olivary nucleus (MSO) of mammals on the basis of morphological characteristics (Boord, '68, '69) and its presumed relation to sound localization (Erulkar, '72). The present electrophysiological observations support this relationship in at least two respects. First, the distinctive laminar organization, with polarized inputs, causes the polarity reversal of an acoustically-driven evoked potential as an electrode traverses the cellular and ventral neuropil layers of n. laminaris. Similar observations have been made in MSO

Galambos et al., '59; Goldberg and Brown, '68; Guinan et al., '72b). Secondly, in a very careful study of response properties of superior olivary complex units, Guinan et al. ('72a,b) found that MSO units were optimally driven by the same frequency applied to either ear and that most units were excited by a tonal stimulus to either ear. The present observations on n. laminaris demonstrate similar characteristics.

A note on morphological terminology

The posterior ramus of the eighth cranial nerve of birds is composed of three separate nerve trunks: the inferior vestibular nerve innervating the crista of the posterior semicircular canal, the macula of the saccule and the small macula neglecta; the cochlear nerve innervating the hair cells of the basilar papilla; and the lagenar nerve innervating the macula lagena (Boord, '69). At present, the function of the macula lagena is not understood. Although it lies at the distal end of the cochlea, there are both morphological and physiological reasons to suspect that its role is not in audition.

The macula lagena resembles a vestibular more than an auditory receptor (Boord, '69) and its innervation pattern and ganglion cell location are distinct from those of the cochlea. In addition, the size range of the axons forming the lagenar nerve is distinctly different from the cochlear portions of the avian eighth nerve (Boord, '69). Turning to their central projections, older studies on normal material reported that the ventrolateral division of n. magnocellularis receives afferents directly from the cochlear ganglion (Holmes, '03; Ramón y Cajal, '08; Sanders, '29). However, Boord and Rasmussen ('63) and Boord and Karten ('74), using degeneration methods following partial destruction of cochlear and lagenar nerve segments, have gathered considerable evidence that the ventrolateral division of n. magnocellularis receives lagenar but not cochlear afferents. These authors conclude that the lateral and medial portions of n. magnocellularis receive little or no innervation from the macula lagena. Furthermore, the termination sites of the lagenar ganglion, falling outside the ventrolateral portion of n. magnocellularis, more closely resemble the projections of the vestibular than the cochlear portion of the eighth nerve (Boord and Karten, '74). Our observations on embryonic and hatchling chicken tissue stained by Nissl, protargol, Golgi-Kopsch and Golgi-Cox methods (Rubel and Parks, unpublished observations) indicate that the ventrolateral division of n. magnocellularis is cytoarchitecturally distinct from both the lateral and medial divisions of n. magnocellularis as well as from n. angularis and n. laminaris (fig. 13). Finally, in the present study no evidence could be found that cells in the ventrolateral division of n. magnocellularis are responsive to auditory stimulation in the 0.1 to 5.0 kHz range.

In light of these findings, it is proposed that the ventrolateral division of n. magnocellularis be renamed the lagenar nucleus (Lag. in figs. 1, 6, 13). A similar suggestion is made by Boord and Karten ('74). Such a step would remove this cell group from its present confusing association with the neighboring, but apparently functionally distinct, cochlear nuclei and would formally recognize its close relationship with the macula lagena.

ACKNOWLEDGMENTS

We would like to express our gratitude to the following individuals: Mr. James E. Cox for the statistical analyses; Mrs. W. Craig for histological processing of the tissue; Mr. Gus Ogren, Mr. Nigel Cox and Mr. Fred Davis for building and maintaining apparatus; Mr. Loring Ingraham, Mr. J. R. H. Jackson, Ms. Laurie Miller, Mr. Daniel Smith, Ms. Peggy Stillman and Mr. Raoul Garcia y Vega for assistance during the recording sessions; Mr. M. Rosenthal for photographic work; Dr. J. I. Johnson, Mr. M. Ostapoff and Mr. H. Weingarten for helpful comments on an earlier draft of the manuscript; and Ms. Lin May for secretarial assistance. The research was supported by grant GB 31934 from the National Science Foundation.

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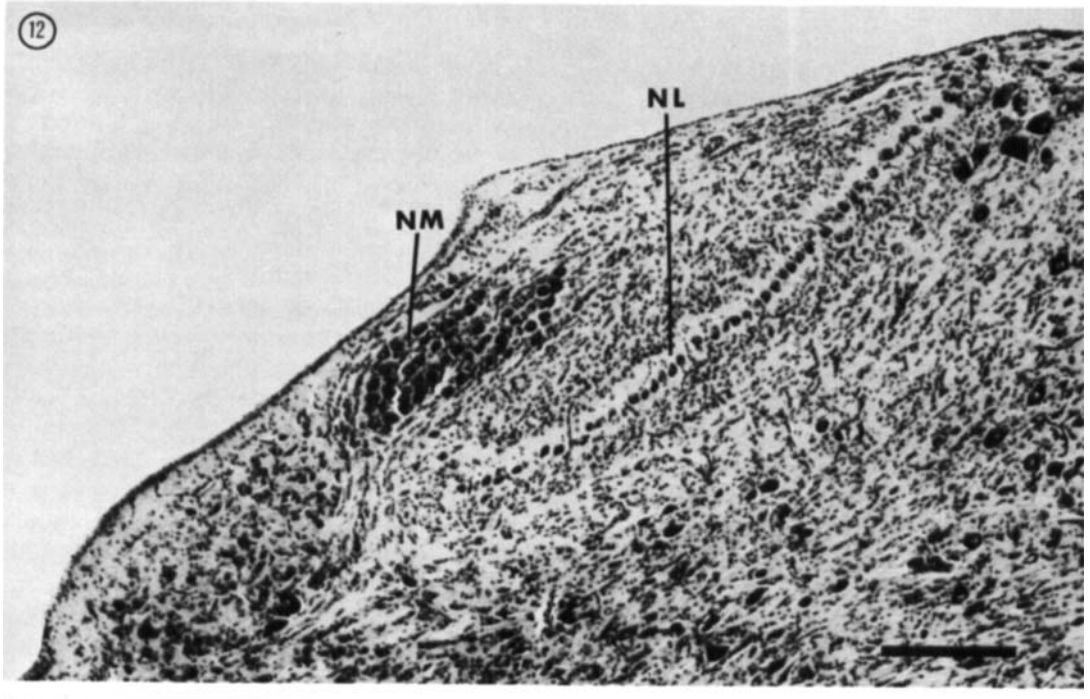
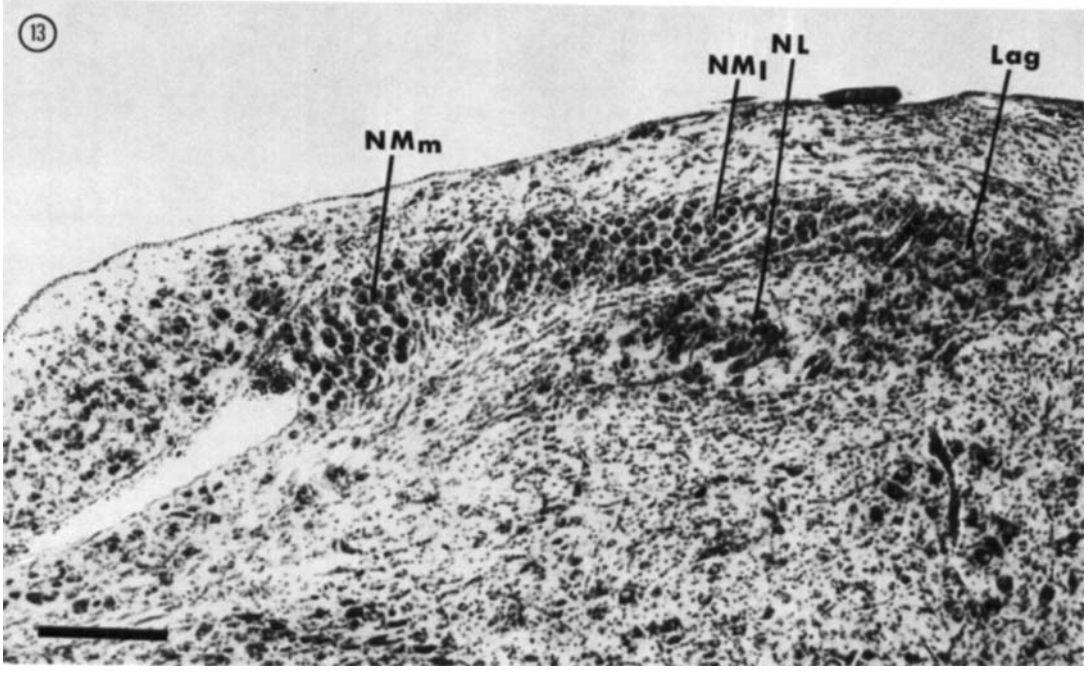
PLATE 1

EXPLANATION OF FIGURES

12 Low-power photomicrograph of thionin stained coronal section through right medulla showing n. magnocellularis, pars medialis (NM) and n. laminaris (NL). Bar indicates 0.2 mm. This section is from approximately the level indicated by no. 45 in figure 1.

13 Low-power photomicrograph of thionin stained coronal section approximately 400 μ caudal of the section shown in figure 12. This section shows: the caudal end of n. magnocellularis, pars medialis (NMm); n. magnocellularis, pars lateralis (NMl); and Lagenar nucleus (n. magnocellularis, pars ventralis lateralis of Boord and Rasmussen, '63; Lag). The extreme caudal pole of n. laminaris (NL) is directly below NM, pars lateralis. Bar indicates 0.2 mm.


12
NL
NM


13
NMl
NL
Lag
NMm