# The Cochlear Nuclei of the Caiman

Soares D*, Simon J[ï] and Carr C*
*Dept. Biology, [ï]Dept. Electrical Eng. Univ. Maryland, College Park
Daphne Soares can be reached at **Daph@wan**

## ABSTRACT

**In the bird and reptilian auditory brainstem, auditory nerve afferents synapse on the neurons of the cochlear nucleus magnocellularis (NM), which in turn project bilaterally to the nucleus laminaris (NL). The ipsilateral axons from NM enter NL dorsally, while the contralateral axons enter it ventrally. These afferent axons form maps of interaural time difference (ITD) in the NL. The organization of the ITD circuit differs among species.**

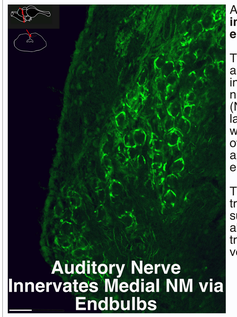
**This variation is possibly associated with the animalìs ability to resolve time differences. Chickens can be considered to have a plesiomorphic pattern. In these birds NL is composed of a monolayer of bipolar neurons which receive input from ipsilateral and contralateral NM forming a single map of interaural time difference in the medio-lateral direction (Young and Rubel, 1983). The barn owl on the other hand, localizes sound accurately and is considered to be a specialist, having a well developed, multilayered NL. The similarities between birds and reptiles are likely due to either the conserved nature of auditory information computation and/or their close phylogenetic relationship. To explore this relationship, we examined the cochlear nuclei of the Caiman (caiman crocodilus) by using immunohistochemical and the Golgi posed of large, round cells where the auditory nerve makes endbulbs on the soma; laterally there is a stellate cell region where punctate terminal endings appear. In NL, bipolar cells created a straight monolayer medially with dense staining on dendrites and cell bodies; laterally a thick layer of compacted cell bodies gave rise to long dendrites that were stained diffusely.A neuron model of coincidence detection was used to investigate the output of NL cells. Caiman parameters from Klinke and Smolders (Hear Res 1986:24/2):89-103) along with measured cell size, dendritic length, thickness, branching and synaptic density were used. Chicken parameters were also used for complementary biophysical standards. Under these circumstances caiman NL neurons responded well to changing ITDs. The same experiments were done at 40 °C and 20°C to investigate the role of temperature change in coincidence detection in these animals.**

## INTRODUCTION

The auditory central nervous system seems to be organized in a pattern common to both birds and crocodilians. These similarities are presumably due to the conserved nature of auditory sense and the close phylogenetic relationship between these groups. Since a great deal is known about the time coding cochlear nuclei in birds it is therefore interesting to further describe the cochlear nuclei of crocodilians.

Crocodilians have a relatively low hearing range (20 - 2,800 Hz). Their ears are large, with a long basilar membrane and unidirectional population of hair cells covered by a tectorial membrane. The auditory nerve projects to two cochlear nuclei: nucleus magnocellularis (NM) and nucleus angularis (NA). In barn owls, the projection to NM preserves the phase-locked response to the stimulus from the auditory nerve via specialized endbulb synapses, while loudness information is preserved in the projection to NA. NM sends bilateral inputs to nucleus laminaris (NL). In birds, NL contains a map of ITDs created by delay line inputs from NM.

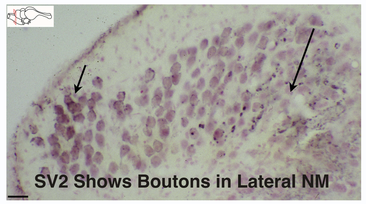
## The Organization of Nucleus Magnocellularis Resembles that of Avians



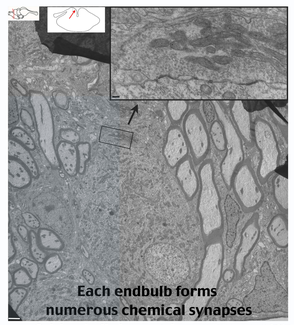

**Auditory Nerve innervates medial NM via endbulb synapses.**

The medial branch of each auditory nerve fiber innervates the cochlear nucleus magnocellularis (NM) and gives rise to the large axosomatic endings which resemble endbulbs of Held. Each neuron appears to receive several endbulb terminals.

The medial branch axons travel on the dorsal surface of the brainstem, above the NM efferent tract. Fascicles descend ventrally to innervate NM.

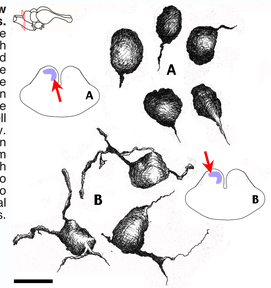



**There are 2 types of projections from the auditory nerve to NM cells.** The distribution of antibodies against synaptic vesicle marker (SV2) throughout the tonotopic axis of NM reveals bouton like projections located laterally (right) to endbulbs. About half of NM neurons received bouton terminals and half endbulbs.




**Each endbulb forms numerous chemical synapses.** These synapses are usually located on the cell body. Endbulb synapses are asymmetric, on the presynaptic side there are usually a small cluster of clear vesicles. Other vesicles of the same size are distributed throughout the cytoplasm of the endbulb. The terminals contain a large number of membranous cisterns of varying sizes, in addition to clathrin-coated endocytotic vesicles. The cisterns are sometimes found on either side of the cluster of presynaptic vesicles, and may sometimes appear fused with the presynaptic membrane.

**Magnocellular neurons have few dendrites.**
Low best frequencies appear to be represented caudolaterally in NM, with high best frequencies represented rostromedially, and intermediate frequencies mapped across the mediolateral extent of the nucleus. When labelled NM neurons were examined, we found that the number of dendrites per cell decreases with increasing best frequency. Golgi impregnated neurons were seen in transverse sections through the brainstem of alligators. Cells in the medial or high best frequency portion of NM tended to have large cell bodies and one or no dendrites, while most cells from the caudal regions had 3 or more dendrites.

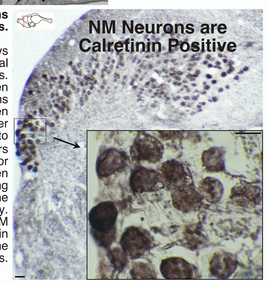



There are 2 Cell forms in NM

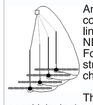
**Horizontal reconstruction shows segments in NM.** The nucleus magnocellularis can be divided into caudal, central and rostral segments. The caudal low best frequency segment was bounded rostrally by the point of entry of the auditory nerve. The central segment (green and blue) can be seen as a long "tear drop" shape visible in transverse sections through the brainstem, while the rostral segment (red and yellow) looks like a column of cells.

**Calcium buffering proteins are present in NM cells.**

Brainstem auditory pathways generally contain several calcium binding proteins. Calretinin has especially been found in systems where neurons phase-lock, and it has been hypothesized that it and other calcium binding proteins act to buffer the $Ca^{2+}$ which enters through glutamate receptor activation. It has also been suggested that calcium binding proteins protect cells from the effects of high rates of activity. The cells of the crocodilian NM and NL are Calretininin immunoreactive, as are the auditory nerve terminals.

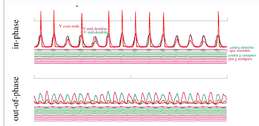



## A NEURON MODEL YIELDS GOOD ITD CODING AT LOW BEST FREQUENCIES



An ITD discriminator neuron should fire when inputs from two independent neural sources coincide (or almos coincide), but not when two inputs from the same neural source (almost) coincide. A neuron that sums its inpu linearly would not be able to distinguish between these two scenarios. This biophysical model uses the progra NEURON to examine an NL neuronìs detection and encoding of ITDs, its mechanisms and their limitations. For the modelìs parameters, dendritic length and width estimates come from Alligator (shown above) and vect strength estimates from Caiman (kindly provided by Jean Smolders and Rainer Klinke). All other parameters a chick [Refs. Rathouz & Trussel, 1998, and Reyes, Rubel, & Spain, 1996].

The model emulates an two dimensional array of neurons, arranged tonotopically in one dimension and by del sensitivity in the other. Each neuron has: a pair of dendrites 20 - 400 mm long (according to tonotopic location), 0.5 - 4 mm in diameter and 30 excitatory synapses; a soma of diameter 15 mm; a myelinated axon with hillock. Ionic channel types include potassium (high and low voltage activated [~Kv1.1, 1.2, 3.1] and delayed rectifier), sodium, and passive. Channel parameter were obtained from physiological studies of chick Nucleus Magnocellularis (NM) and NL [Refs. Rathouz & Trussel, 1998, and Rubel, & Spain, 1996]. Each neuron in the array feeds into a single inhibitory neuron, which feeds back onto all neurons in the All synapses fire with conductance proportional to an alpha-function. The excitatory synapses fire as individual Poisson proce with probability rate given by a modified sinusoid, with adjustable amplitude and vector strength. The stimulus is a pure tone adjustable frequency, with adjustable interaural phase difference.

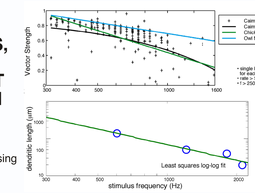



### NEURON SIMULATION REVEAL GOOD CODING FOR IN PHASE STIMULI

A pair of cells receives the same stimulus probability distributions (here, f Hz). The top receives its inputs binaurally in-phase, and the bottom out-o Red tracks the intracellular potential at the axon s first node of Ranvier, n in the ipsilateral dendrite center, and green in the contralateral dendrite c Below them are the excitatory inputs, both in probability and conductance (for some of the 60 synapses).

### AT LOW BEST FREQUENCIES, PHASE LOCKING IN THE CAIMAN IS SIMILAR TO THAT OBSERVED IN THE CHICKEN

Like the chicken, the alligator and caiman NL neurons show a decrease in dendritic lenght with rostrocaudal position in NL.All trials co-vary the dendritic length and stimulus vector strength with the stimulus frequency, using experimentally derived relations.




A linear fit betw vector strength frequency, dec 90% to simulat instead of AN.

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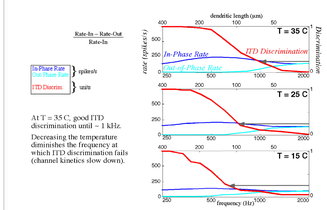
### Temperature has an effect on ITD




At T = 35 C° good ITD discriminations until ~ 1 kHz.

Decreasing the temperature diminishes the frequency at which ITD discrimination fails (channel kinetics slow down).

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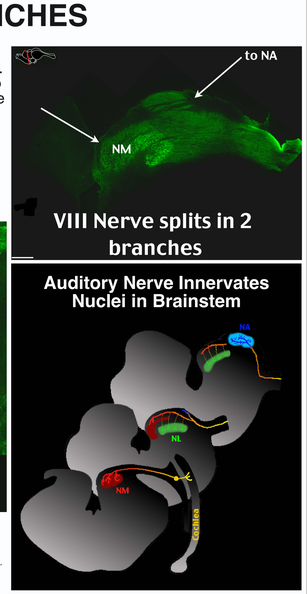
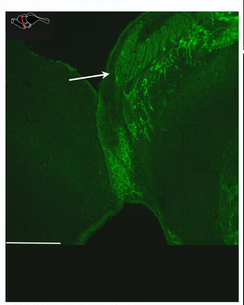
## AUDITORY NERVE HAS 2 MAJOR BRANCHES

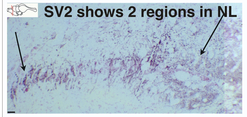
The auditory nerve enters the brainstem and forms a thick sheet on its dorsal surface. The entry point is caudal to NA and dorsal to NM. The nerve then splits in 2 branches. The rostral branch projects to NA. The caudal branch subdivides to form many branches which innervate NM. In two cases where cholera toxin was used as a tracer, some auditory nerve collaterals descended into NL where they synapsed upon the dorsal dendrites of the NL neurons.



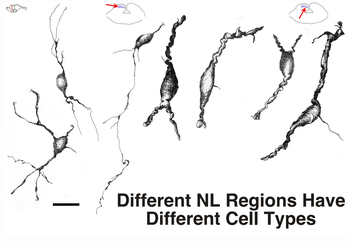



**METHODS FOR COCHLEAR INJECTION**
Hatchling Alligator mississipiensis were anesthetized. Animals were placed on iced water ventral side up on a small dish. A small incision was made on the ventral side of the jaw. Muscles were moved apart until the roof of the head was seen. A small probe was used to open a whole where the cochlea and the laguena are localized. In one case the cochlea was completely removed and on the other is was damaged. Cholera toxin (List labs) was injected on the site and Gelfoam was used to seal the hole. 2-3 sutures were made and animals were kept in a warm tank for 11 days. Animals were perfused with 0.9% NaCl followed by 4% Paraformaldehyde in 0.1MPhosphate Buffer. Traditional immunohistochemical procedures were followed for fluorescence and chromagen techniques.

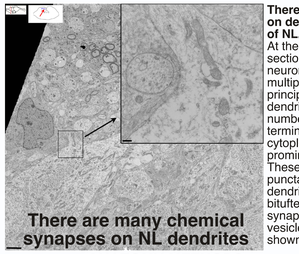
## NUcleus LAMINARIS in CROCODILIANS HAS TWO REGIONS




**Two distinct regions can be seen in NL.**
Rostromedial NL is characterized by a monolayer of bitufted neurons which receive large SV2 labeled boutons on both dendrites and cell bodies. This region may be associated with the detection of higher CF.
Caudolaterally, synapses marked by SV2 are segregated to the long dendrites of the bitufted cells. Cell bodies are longer found in a monolayer but a compact layer with many somata. Synapse density was sparser in this region.

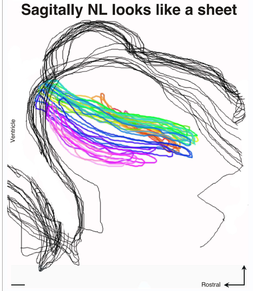



**More than one cell type are present in NL.** Cell types in the NL were characterized from 5 Golgi-impregnated brains and 2 transynaptic choleratoxin backfills. Like NM, NL we found 2 different cell types in NL. One type is resembled the typical low best-frequency bipolar neuron observed in the chicken adn was localized on the high frequency regions. The other cell type was characterized by long, thing dendrites and smaller cell bodies. This type was confined to the lateral most area of NL,and its dendrites contributed to the neuropil at the dorsal tip of the caudolateral NL.

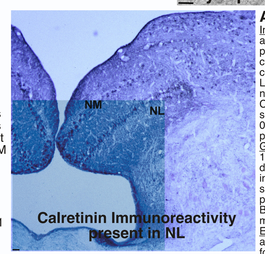



**There are chemical synapses on dendrites of both divisions of NL.**
At the ultrastructural level, sections through NL contained neurons with a characteristic multipolar form and large principal dendrites. These dendrites received large numbers of synapses from terminals characterized by pale cytoplasm, round vesicles and a prominent postsynaptic density. These terminals formed small punctate synapses on the dendrites and cell bodies of the bitufted NL neurons. Another synaptic type with larger round vesicles was also observed (not shown).

### Sagitally NL looks like a sheet




**3D reconstruction shows NL as a flat layer.**
The nucleus laminaris (NL) is an flat sheet when in the sagittal plane. It is an oval shaped nucleus in the horizontal plane except at its most caudal extent where it forms a bulge at its lateral edge. This lateral bulge contains distinctly smaller bitufted cells with longer dendrites than in the most medial part of NL. The nucleus is surrounded by fibers from NM. The dorsal border of the nucleus is composed of afferents from the ipsilateral NM and possibly afferent aons from the auditory portion of the VIII nerve (see left panel). NM afferents are overlaid by auditory nerve axons in the medial and caudal parts of the nucleus laminaris. The ventral border of the nucleus laminaris consists of fibers from the contralateral NM. All Luminaris neurons have oval cell bodies, with their long axis oriented parallel to the dorsoventral axis of the nucleus. We assume that rostral NL receives high best frequency input (above 1 kHz) and that caudal NL receives lower best frequency inputs. In birds, NL neurons act as coincidence detectors, and receive inputs from ipsilateral NM onto their dorsal dendrites and inputs from contralateral NM onto their ventral dendrites. In chickens, these NM axons act as delay lines to create a map of interaural time differences (ITD) along the mediolateral extent of NL. A similar map could exist in the crocodilian, at least for air-borne sound.




All neurons in nucleus laminaris appear to be intensely Calretinin immunoreactive.

### ANATOMY METHODS

Immunohistochemistry: Alligators and Caiman were anesthetized as above and perfused transcardially with normal saline, followed immediately by 4% paraformaldehyde in 0.1M phosphate buffer. Standard immuno-cytochemical procedures were followed using the avidin-biotin-peroxidase complex (ABC) method with reagents from Vectastain elite kits (Vector Labs). Sections were pre-incubated for 20 minutes in 0.1M PBS with 4% normal serum and 0.4% Triton-X, then incubated overnight in either SV2 or Calretinin antibody. Sections were incubated for 1 hr in biotinylated IgG secondary, diluted 1:1500, in ABC for 1 hr, than reacted for 10-20 min with 0.04% diaminobenzidine tetrahydrachloride (DAB) and 0.003% hydrogen peroxide in Tris-imidazole buffer.

Golgi:A Golgi-aldehyde technique was used on 28 crocodilians (Valverde, 1970). Hatchlings were anesthetized with Ketamine, followed by a lethal dose of Nembutal (10 mg/kg i.m.; Abbott Laboratories). After intracardiac injection of heparin, animals were perfused transcardially with normal saline, followed immediately by 1 liter of 4% paraformaldehyde in 0.1M phosphate buffer at pH 7.4. The brains were then placed in Golgi fixative. Brains were sectioned transversely at 90 µm thickness using a sliding microtome.

Electron Microscopy: For ultrastructural studies, 2 alligators were anesthetized as above and perfused transcardially with Billing's solution, followed by 3% glutaraldehyde and 1% paraformaldehyde in 0.1M phosphate buffer at pH 7.4. The brains were postfixed overnight, then sectioned in the transverse plane. Selected regions were postfixed with 1.0 % osmium tetroxide. Following postfixation, the tissue was dehydrated and embedded in Polybed 812 resin. Before and after each series of thin sections, several 1-2 µm semithin sections were cut and stained with toluidine blue. Thin sections were stained with uranyl acetate and Reynold's lead stain, and examined with a Zeiss electron microscope.

## CONCLUSIONS

**The organization of the hindbrain auditory nuclei in crocodilia appears to be similar to that in birds**
**The auditory nerve innervates nucleus magnocellularis (NM), nucleus angularis (NA) and possibly the dorsal region of nucle laminaris (NL)**
**Auditory nerve terminals form endbulbs in rostromedial NM a boutons in caudolateral NM**
**NM are calretinin immunoreactive. Rostrally, cell have round c bodies and few or no dendrites, while caudal cells have more dendrites.**
**NL can be subdivided in 4 regions with characteristic cell type**
**A combination of crocodilian and chicken parameters in a neu model yielded good ITD coding at low best frequencies**

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