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Nucleus Laminaris

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Our ability to detect subtle acoustic cues in noisy environments, like a conversation in a crowded restaurant, is one benefit of binaural hearing. Binaural hearing is also essential for sound localization. The ability to localize the source of a sound is dependent on time disparities in the arrival of low-frequency signals between the two ears, referred to as interaural time difference (ITD). In all vertebrates, ITDs are encoded by distinct neural circuits in the central auditory nervous system specialized for the temporal processing of sound at the network, synaptic, and cellular levels. Coding of ITDs is first performed by the medial superior olive (MSO) of mammals and by the nucleus laminaris (NL) in birds and some reptiles.

This chapter will focus on the microcircuitry of the chicken NL, which is an excellent example of neural architecture exquisitely tailored for its specialized function in sound localization. Neurons in NL are coincidence detectors, encoding temporal information of sound arriving at the two ears by responding maximally when resulting action potentials (APs) arrive simultaneously, a unique feature responsible for the coding of ITDs in the microsecond range. We will discuss the important structural and functional specializations of NL that optimize this specialized ability, fundamental for binaural hearing in most birds and mammals.

Specialized Features of Nucleus Laminaris

Nucleus laminaris neurons are bipolar; dendrites extend dorsally and ventrally from the soma to form two segregated dendritic domains (Fig. 22.1A). Cell bodies of NL neurons align into a single sheet, resulting in separate

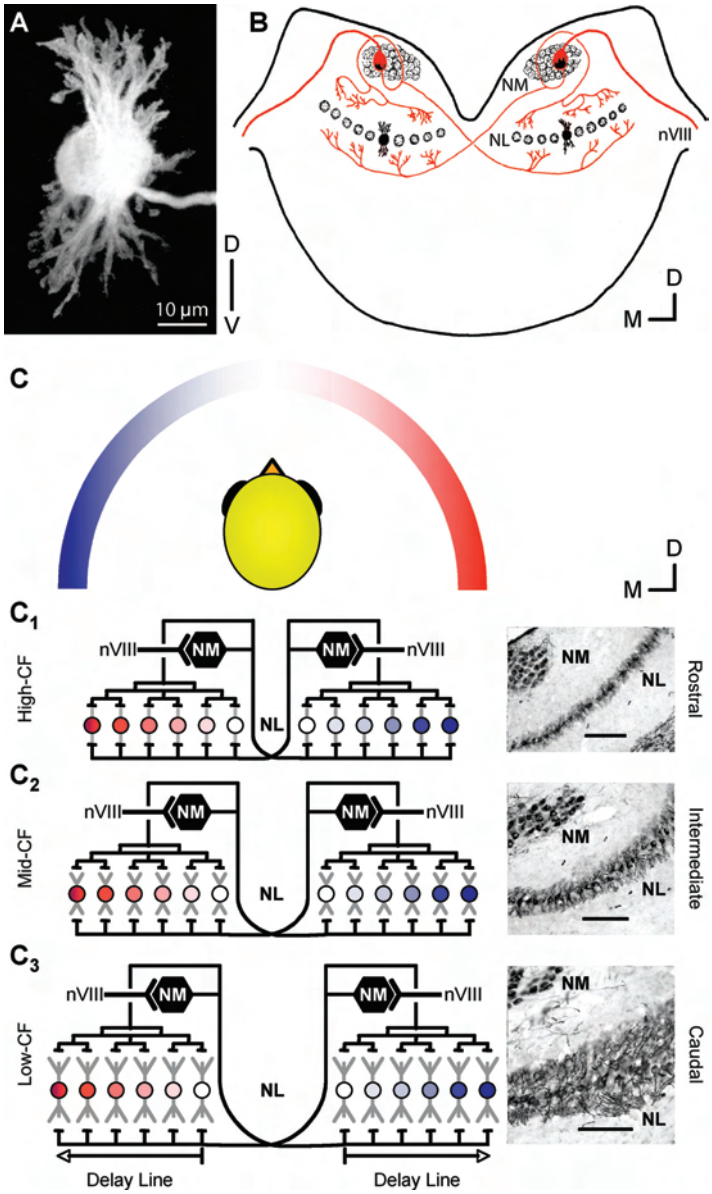

Figure 22–1. Specialized features of nucleus laminaris (NL). (*A*) Bipolar NL neuron filled with Alexa 488 clearly shows dorsal (D) and ventral (V) segregated dendrites. (*B*) Schematic drawing of the organization of excitatory inputs (red lines) to NL (coronal view). The dorsal and ventral dendritic domains of NL neurons receive glutamatergic inputs from the ipsilateral and contralateral NM, respectively. The axon lengths to the dorsal NL are equal along an iso-frequency dimension, while the contralateral axon to the ventral NL produces a delay line that compensates for interaural time delays (M, medial; D, dorsal; nVIII, eighth cranial nerve). (*C*) Schematic representation from (*B*) (modified with permission from Dr. Armin Seidl). Blue-white-red arc represents different sound source positions along the horizon. NL neurons respond best to the sound source of the same color and maintain this topographic representation of interaural time delays across iso-frequency lamina ($C_{1–3}$). Sounds arriving from straight ahead are encoded by NL neurons located medially (white), while sounds originating from the left (blue) and right (red) sides are encoded by neurons located most laterally from the contralateral NL. Note that the three schematics include NL neurons with differing dendritic lengths. Representative MAP2 immunoreactivity sections of NL from the rostral (high-CF), intermediate (mid-CF), and caudal (low-CF) regions are shown in the far right panel to show the dendritic gradient. Scale bars, 100 µm. (From Wang and Rubel, 2008)

dorsal and ventral dendritic neuropil laminas. Structurally, the bipolar configuration and single-cell body layer set the ground rules for the organization of afferent inputs, which is critical for coincidence detection in binaural hearing. Functionally, several physiological mechanisms account for coincidence optimization and are due in part to both intrinsic and synaptic properties of NL neurons. Several key anatomical and physiological mechanisms are highlighted in this chapter.

GLUTAMATERGIC EXCITATORY INPUTS

Nucleus laminaris receives glutamatergic excitatory inputs solely from the nucleus magnocellularis (NM), a primary cochlear target, on both sides of the brain. This projection is arranged in a strict topographic manner, resulting in a precise map of sound frequencies (tonotopic) in NL (Rubel and Parks, 1975). That is, NL neurons responsible for encoding high-frequency information are located in the rostro-medial pole, and neurons that are optimally activated by lower frequency acoustic events are positioned progressively in the caudolateral region of the nucleus. Ipsilateral and contralateral terminals from the same parent neuron in NM target identical tonotopic positions in NL but segregate onto dorsal and ventral dendritic domains, respectively (Fig. 22.1B). These terminal arbors are highly anisotropic; oriented orthogonal to the tonotopic axis and confined within a specific iso-frequency band (Young and Rubel, 1983). With this arrangement, individual NL neurons receive information about acoustic signals at the same specific sound frequencies from both ears.

Binaural hearing is further enhanced by substantial convergence and divergence of NM innervation on NL neurons within the same iso-frequency band. In general, NM terminals converge onto about 42% of the somatic surface and 63% of the dendritic surface of NL neurons, as well as the axon hillock and initial segment (Parks et al., 1983). The axon of individual NM neurons appears to terminate onto 20–35 NL neurons, and the composite character of excitatory postsynaptic potentials (EPSPs) by intracellular recordings suggests that multiple NM axons converge onto each NL neuron (Hackett et al., 1982). These integrations occur within iso-frequency bands and thus maintain tonotopic specificity.

To perform coincidence detection between the two ears, ipsilateral and contralateral terminal arbors of NM neurons form radically different and highly stereotyped morphologies. The ipsilateral axon bifurcates to provide equivalent axon length to dorsal dendrites of NL neurons along an iso-frequency dimension (Young and Rubel, 1983). The contralateral axon, however, from NM neurons in the same tonotopic position extends across the midline and bifurcates several times to create an orderly, serial set of axonal branches to ventral dendrites along a matching iso-frequency lamina of NL on the opposite side of the brain. This arrangement of innervation,

predominately along the medial to lateral dimension, results in medial NL neurons receiving contralateral inputs from the shortest input axons and lateral NL neurons receiving the longest axons. This systematic increase in axonal length across the ventral dendritic field effectively establishes a series of delay lines that compensate for time delays between the two ears and is an excellent example of the modified Jeffress model for processing ITDs (Fig. 22.1C) (Jeffress, 1948).

In concert with these specialized structural features, physiologic properties of individual NL neurons also play a key role in coding time delays between the two ears, improving coincidence detection and ITD processing. One such mechanism involves both pre- (NM) and post- (NL) factors that mediate the depression of synaptic transmission. These factors include the following: the depletion of releasable neurotransmitter vesicles; inactivation of presynaptic Ca^{2+} channels; and the desensitization of postsynaptic AMPA-receptors (AMPA-Rs). In NL, where intrinsic and synaptic responses are extremely brief in order to accommodate rapid transmission, excitatory postsynaptic currents (EPSCs) help control the time course of EPSPs, thus minimizing distortion (Trussell, 1997, 1999).

For example, evoked AMPA-R-mediated EPSCs in NL are extremely fast, having an average decay time constant of less than 1 ms (Fig. 22.2A), due in part to the expression of the GluR3 and GluR4 subunits. Furthermore, there is a considerable reduction in EPSC amplitudes following a train of stimuli (Fig. 22.2B). This synaptic depression (i.e., the reduction of glutamatergic neurotransmitter released from NM) plays a key role in improving coincidence detection in NL (Funabiki et al., 1998). As first reported by Funabiki et al. (1998), a positive correlation exists between the optimal response window for coincidence detection and the EPSP decay time constant. However, the observed importance of EPSP amplitude, namely, the response window obtained by subthreshold stimuli was narrower than those obtained using intense stimuli. This observation was later confirmed to be critical for coincidence detection and ITD processing (Kuba et al., 2002). Using a stimulus train at a fixed intensity and frequency, Kuba et al. (2002) reported significantly reduced EPSPs that corresponded to a decrease in the width of the response window for coincidence detection. Consequently, the accuracy of coincidence detection is improved not only by the faster decay time course of evoked responses but also by the smaller amplitudes, reducing the confounding effects of stimulus-intensity-related information (Cook et al., 2003).

In addition to the aforementioned synaptic mechanisms, the extremely fast EPSP of NL neurons are also due to specialized intrinsic properties. Namely, the expression of strong voltage-activated K^+ conductances (K^+_{VA}), which consists of high-threshold and low-threshold K^+ channel currents (K^+_{HVA} and K^+_{LVA}, respectively). The expression of the Kv3.1 channel is responsible for the K^+_{HVA} currents and is activated at depolarizing potentials (~ −20 mV), while the Kv1.1 channel is responsible for K^+_{LVA} currents, which are

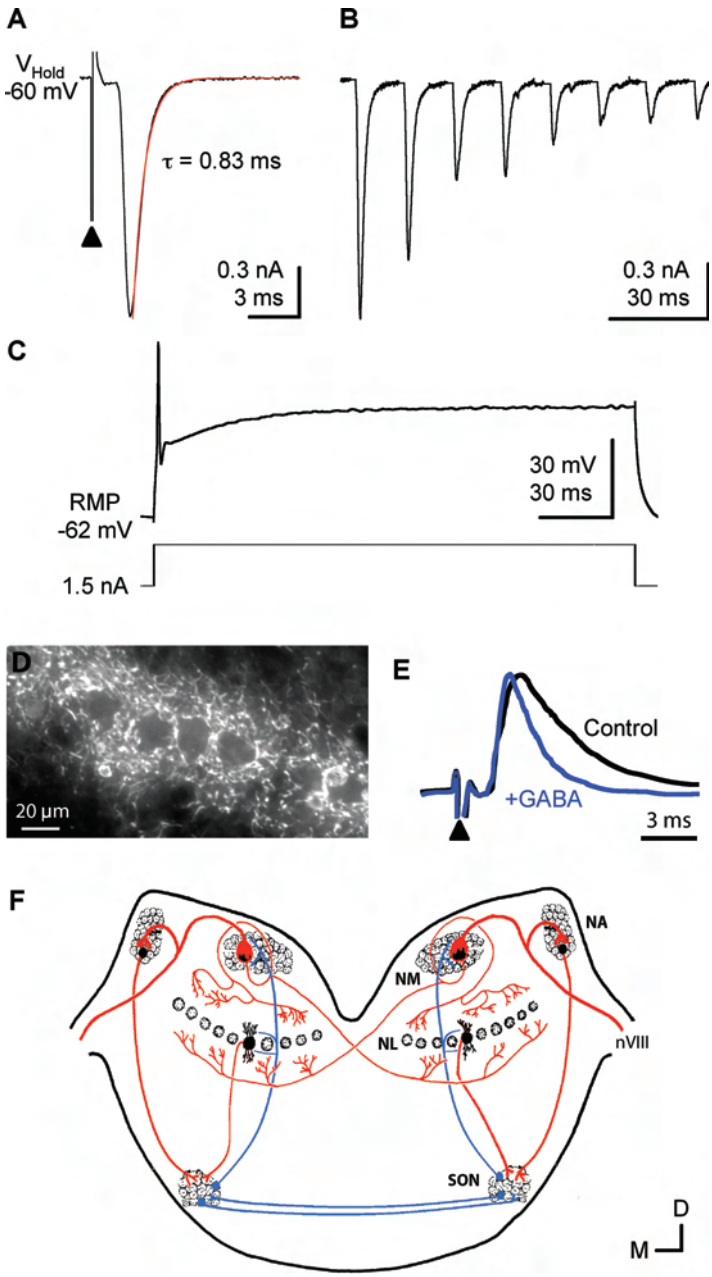

FIGURE 22–2. Specialized features of nucleus laminaris (NL). (*A*) Voltage-clamp recordings showing the decay time constant of an isolated AMPA-R evoked excitatory postsynaptic current (τ, tau). τ was fit with an single exponential (superimposed red-trace). (*B*) Synaptic depression in NL following an 80 Hz stimulus train. V_{Hold} = –60 mV for traces in (*A*) and (*B*). (*C*) Current-clamp recording (top trace) of a NL neuron showing a single AP following a prolonged current injection into the soma (1.5 nA, 100 ms, bottom trace). RMP, resting membrane potential. (*D*) GABA immunoreactivity on both the soma and dendrites of NL neruons. (*E*) Local GABA application (10 µM) shortens the excitatory postsynaptic potential (EPSP)

Figure 22–2. continued
decay time constant (blue trace) compared to control EPSP (black trace). Arrowheads in (*A*) and (*E*) indicate stimulus artifact. Stimulus artifacts in (*B*) removed for clarity. Normalized traces in (*E*) are modified with permission from Funabiki et al. (1998). The rapid AMPA-R kinetics (*A*), synaptic depression (*B*), single AP firing (*C*), and GABAergic depolarization (*E*) are just a few examples of how NL neurons optimize coincidence detection. (*F*) Schematic drawing of the inhibitory feedback loop (coronal view). Red and blue lines indicate excitatory and inhibitory projections, respectively. The reciprocal connections between superior olivary nuclei (SONs) are believed to preserve interaural time difference (ITD) processing in NL by equalizing input strength from nucleus magnocellularis (NM). For the purpose of the clarity, projections from SON to nucleus angularis (NA) and ascending projections to more rostral regions of the brain are not included.

activated at or near the resting membrane potential. The strong expressions of these K^+ channels have several important influences on the function of NL neurons acting as coincidence detectors. First, K^+_{HVA} conductances allow neurons the ability to repolarize quickly following the generation of an AP. Second, strong outward K^+_{LVA} conductances reduce input resistance while increasing the membrane time constant, thus shortening the time window inputs can summate. Finally, this K^+_{LVA} conductance increases the neurons threshold for AP generation and as a result, NL neurons elicit only a brief, single AP at the onset of a prolonged current injection (Fig. 22.2C) (Reyes et al., 1996). Thus, only strong excitatory inputs excite NL neurons. Taken together, fast AMPA-R kinetics, significant synaptic depression, and the strong expression of K^+ channels allow NL neurons to be highly sensitive to simultaneous bilateral inputs from NM, a required prerequisite for coincidence detector neurons and for optimizing ITDs.

Furthermore, the distribution of the site of AP generation in NL neurons is specialized for the accuracy of ITD detection. In NL, the site of AP initiation in the axon is arranged at a distance from the soma and is dependent on the frequency tuning of the neuron. That is, in high characteristic frequency (CF) neurons, Na^+ channels are located 20–50 μM from the soma, while low-CF neurons have Na^+ channels clustered in longer segments of the axon closer to the soma. This distribution of Na^+ channels in NL has functional benefits: first, APs are initiated at more remote sites as the CF of NL neurons increase, optimizing ITD processing at each CF; second, the reduction of Na^+ channel inactivation is crucial in detecting ITDs with accuracy during high-frequency inputs (Kuba et al., 2006).

GABAergic Inhibitory Inputs

In addition to the glutamatergic excitatory inputs from NM, NL neurons are innervated by GABAergic inhibitory terminals, which cover about 31% of the

somatic surface and 10% of distal dendritic surface of NL neurons (Parks et al., 1983). The major origin of this inhibitory input is from GABAergic neurons located in the superior olivary nucleus (SON). In contrast to NM inputs, the projection from SON to NL apparently lacks precise terminal arbor specificity related to the tonotopic organization of NL, suggesting that the activity of this projection is not highly tuned to sound frequency.

The role of SON input on coincidence detection of NL neurons underlies the general pattern of SON innervation with a number of auditory nuclei involved. First, SON receives excitatory inputs from the ipsilateral NL and in turn projects back to NL and NM on the same side, forming an inhibitory feedback loop (Yang et al., 1999). Second, this loop is accompanied by two additional inputs to SON, one from the ipsilateral nucleus angularis (NA), and the other from the contralateral SON, which provides a negative coupling between feedback loops on each side of the brain (Fig. 22.2F). The ipsilateral circuitry is thought to maintain physiological function near a neurons threshold, while the contralateral circuitry is believed to provide a cellular substrate that preserves ITD processing in NL by equalizing input strength from NM (Burger et al., 2005).

Indeed, inhibitory inputs from SON to NL plays a critical role in preserving the stability of ITD processing across a broad dynamic range of sound intensity by providing one of the most unique and uncommon properties in all of the central nervous system. $GABA_A$-receptor ($GABA_A$-R) activation results in a depolarizing response in NL neurons due to unusually high internal Cl^- concentration that persists into maturity. GABAergic depolarization activates K^+_{LVA} conductances, which, as mention previously, lowers the membrane input resistance and shortens the EPSP decay time constant (Fig. 22.2E). This GABAergic effect is attributed to the shunting conductances of postsynaptic $GABA_A$-Rs (Funabiki et al., 1998). This shunting mechanism inactivates voltage-gated Na^+ channels, increasing the threshold for AP generation and provides yet another unique mechanism for optimizing coincidence detection. Taken together, inhibitory inputs enhance intrinsic membrane properties and provide binaural control of system gain for NL neurons.

Dendritic Gradient of Nucleus Laminaris Neurons

The contribution of dendrite geometry for the functioning of single neuron subtypes has remained elusive despite the fact that it is widely accepted that dendritic form plays an important role in neuronal computation. The unique dendritic structure of NL neurons, as well as their known functional role as coincidence detectors, provides an ideal circuit for understanding the specific role of such dendrites in neuronal computation.

In addition to their bipolar configuration, another specialization of NL structure is its highly stereotyped dendritic gradient (Fig. 22.1C) (Smith and

Rubel, 1979). This gradient is evident not only in the total dendritic branch length of individual neurons but also in the distance from the most distal dendritic branches to the soma, that is, the width of the dendritic band. The dendritic gradient conforms precisely to the tonotopic axis with an 11-fold increase in total dendritic branch length and approximately 5-fold increase in the width of the dendritic band from high- to low-CF neurons. The fact that dendritic length increases with decreasing frequency of the optimal sound stimulus suggests a computational role for the dendrites. In contrast, the soma of NL neurons does not show any such gradient.

This dendritic length gradient in NL appears to be an adaptation for ITD processing for particular sound frequencies. Low- and mid-CF neurons, which contain the longer dendrites, may exhibit dendritic filtering resulting from their large surface area (Kuba et al., 2005). A possible advantage of this filtering property is that it may enhance the electrical isolation of dorsal and ventral dendrites and, thus, the inputs from each ear. Indeed, using basic biophysical modeling of known electrophysiological and structural features of NL neurons, recent studies have demonstrated that dendrites improve coincidence detection by allowing a nonlinear summation between the segregated inputs from the ipsi- and contralateral NM (Agmon-Snir et al., 1998). Furthermore, dendrites act as current sinks for each other and modulate the nonlinear integration of inputs. Their results confirm that one aspect of the unique morphology of NL neurons, the spatial segregation of the inputs to different dendrites, enhances the computational power of these neurons to act as coincidence detectors. However, the precise role of this 11-fold gradient in dendritic branch length remains to be convincingly shown.

Developmental Specializations

Chickens can hear well before they hatch (about 21 days of incubation) and the major developmental events in the auditory system occur in ovo. In fact, most if not all, intrinsic and synaptic properties appear mature at the time of hatching with minimal refinement thereafter. The development of these highly specialized physiological, organizational, and morphological features of the NM-to-NL projection is temporally correlated with the establishment of synaptically driven neuronal activity (Jackson et al., 1982) and may be dynamically sculpted by synaptic input. However, NM is not a passive receiver of extrinsic influences; the topography and organization of NM projection onto NL are determined by cues intrinsic to the nucleus. Early unilateral destruction of the otocyst (embryonic precursor of the inner ear) induces formation of a functional aberrant axonal projection to the ipsilateral NM from the contralateral NL, which maintains the tonotopic map in NM and NL on both sides of the brain. In addition, following cochlea removal, the deinnervated NL dendritic domains are innervated by the afferents from the

opposite NM, which are normally restricted to the opposite domain of NL dendrites (Rubel et al., 1981).

Similar to the development of NM terminals in NL, dendritic growth and dendritic gradient in NL are affected by both intrinsic and extrinsic cues. The formation and sharpening of the dendritic gradient is temporally correlated with the onset and maturation of auditory function. Although dendritic length in NL is dramatically affected by synaptic inputs from NM, the dendritic gradient is preserved and appears to be determined by intrinsic properties as well (Parks, 1981). Recent studies have shown that the segregation of inputs to NL can be partially altered by disrupting expression of single genes such as Eph receptors (Cramer et al., 2006), but the basic pattern seems to remain intact, suggesting regulation by multiple pathways.

SUMMARY AND CONCLUSION

The brain develops specialized neurons and circuits to perform particular functions. In birds, NL neurons are part of a circuit responsible for sound localization. The timing challenges of this task, resolving microsecond differences in the arrival time of sound to the two ears, has imposed important constraints on the morphophysiology of the circuitry. Neuronal architecture, dendritic morphology, and afferent organization determine how information is received, while intrinsic and synaptic properties define how the neurons respond to binaural sound information. The chicken NL is an excellent example of a highly specialized brain region where the reasons for these specialization can be understood in terms of the functions the system subserves.

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