Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 8400–8404, November 1986
Neurobiology

Neural map of interaural phase difference in the owl's brainstem

(nucleus laminaris/sound localization/delay line/coincidence detection)

W. E. SULLIVAN* AND M. KONISHI

Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

Contributed by M. Konishi, July 14, 1986

ABSTRACT **Neurons of the barn owl's (*Tyto alba*) nucleus laminaris, the first site of binaural convergence, respond in a phase-locked fashion to a tone delivered to either ear. It may take longer to elicit phase-locked spikes from one ear than from the other. This disparity in delay differs from neuron to neuron and is independent of tonal frequency. In binaural stimulation, neurons respond best when sound in one ear leads that in the other by an amount equal to their delay disparities but opposite in sign. This condition causes simultaneous arrival of phase-locked spikes from the two sides. Laminaris neurons can thus be described as coincidence detectors. The phase of a tone-induced evoked potential, termed "neurophonic," varies systematically with position in nucleus laminaris. From dorsal to ventral within the nucleus, the phase delay of a contralaterally elicited potential decreases and that of its ipsilateral counterpart increases. Therefore, if the neurophonic delay is due to the delay of phase-locked spikes, an orderly representation of delay disparities is shown. Because they act as coincidence detectors, laminaris neurons should show selectivity for interaural phase difference based on their place in the nucleus. Thus, nucleus laminaris presumably measures and maps interaural phase differences by using the principles of delay lines and coincidence detection.**

Sound localization requires neuronal analysis of binaural cues. The "place theory" of sound localization, proposed originally by Jeffress (1), explains how the auditory system can measure interaural time differences, an important cue for horizontal localization in the barn owl (2). The Jeffress model incorporates the concepts of neural delay lines and coincidence detectors. Binaural neurons form an array along which monaural inputs from the two sides converge. The position of a neuron relative to the ends of the array determines the axonal path length from each side. This, in turn, determines the sign and magnitude of the difference in neural transmission delays from the two sides. If the neuron fires maximally when the neural signals from the two sources arrive simultaneously, it would be most sensitive to an interaural time difference that offsets this difference in neural transmission delays. Thus, a map of neural sensitivity to interaural time difference is generated. Several lines of evidence suggest that this model applies to the owl's nucleus laminaris.

MATERIALS AND METHODS

Barn owls (*Tyto alba*) were prepared for electrophysiological recording under Ketamine anesthesia. Glass-coated platinum/iridium electrodes were used for single and multiunit recordings. Electrodes were placed stereotaxically. A PDP 11/40 computer recorded the time of occurrence of amplified and level-discriminated action potentials and stored multiunit potentials digitized at a sampling rate of 25 kHz.

Earphones inserted in the external auditory meatus delivered stimuli that consisted of tone bursts 100 msec in duration with a rise and decay time of 5 msec. The earphones were calibrated for phase and frequency response with a 1.27-cm Bruel & Kjaer microphone. Errors in phase measurement due to variation in earphone placement did not exceed 5 μsec.

Owls were killed with an overdose of sodium pentobarbital, exsanguinated with saline, and fixed with 10% formalin. Relevant brain areas were cut at 30 μm on a freezing microtome, and the sections were stained with cresyl violet. Electrolytic lesions (5 μA, 10 sec, electrode negative) marked some of the recording sites and other reference locations. When an owl was used more than once, the skull opening was filled with dental cement and the skin incision was sutured. The wound edges were coated with Xylocaine jelly for local analgesia and Neosporin ointment for protection against infection.

RESULTS

Single Neuron Recording. Although single neurons were difficult to isolate within nucleus laminaris, their output fibers could be individually studied (3). Analysis of 15 fibers revealed the following neurophysiological attributes. Fibers could be driven by either monaural or binaural stimuli. The same frequency was optimal for ipsi- and contralateral ear stimulation. Action potentials in these fibers tended to occur at or near a particular phase angle of a tonal stimulus—i.e., the spikes are phase-locked. A laminaris fiber phase-locked to a tone delivered to either ear. The preferred phase angle of a fiber differed depending on the side of stimulation (Fig. 1). In Fig. 1, comparison of the left and right period histograms shows a difference of ≈40 μsec (left leading right) for all three stimulus frequencies. This phase difference, termed here "binaural delay disparity," appeared to be unique to a neuron and independent of tonal frequency.

In addition to responding to monaural stimuli, laminaris fibers were sensitive to interaural time difference in binaural stimuli. The time difference eliciting a maximal response in a fiber was equal in magnitude to its binaural delay disparity but opposite in sign. For example, the neuron in Fig. 1 responded maximally when the stimulus in the right ear led that in the left ear by about 40 μsec. Inspection of the monaural period histograms shows the two monaural responses will be in phase if the response to right ear stimulation is advanced by 40 μsec. Thus, a maximal binaural response occurred when the phase-locked spikes from the two sides arrived simultaneously. The interaural time difference that is maximally effective at all frequencies is the characteristic delay of a neuron (4, 5).

The response of a laminaris fiber to variation in interaural phase difference was not all or nothing but was inversely proportional to the amount of deviation from the neuron's optimal phase difference. A laminaris fiber responded max-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*Present address: Department of Biology, Princeton University, Princeton, NJ 08544.

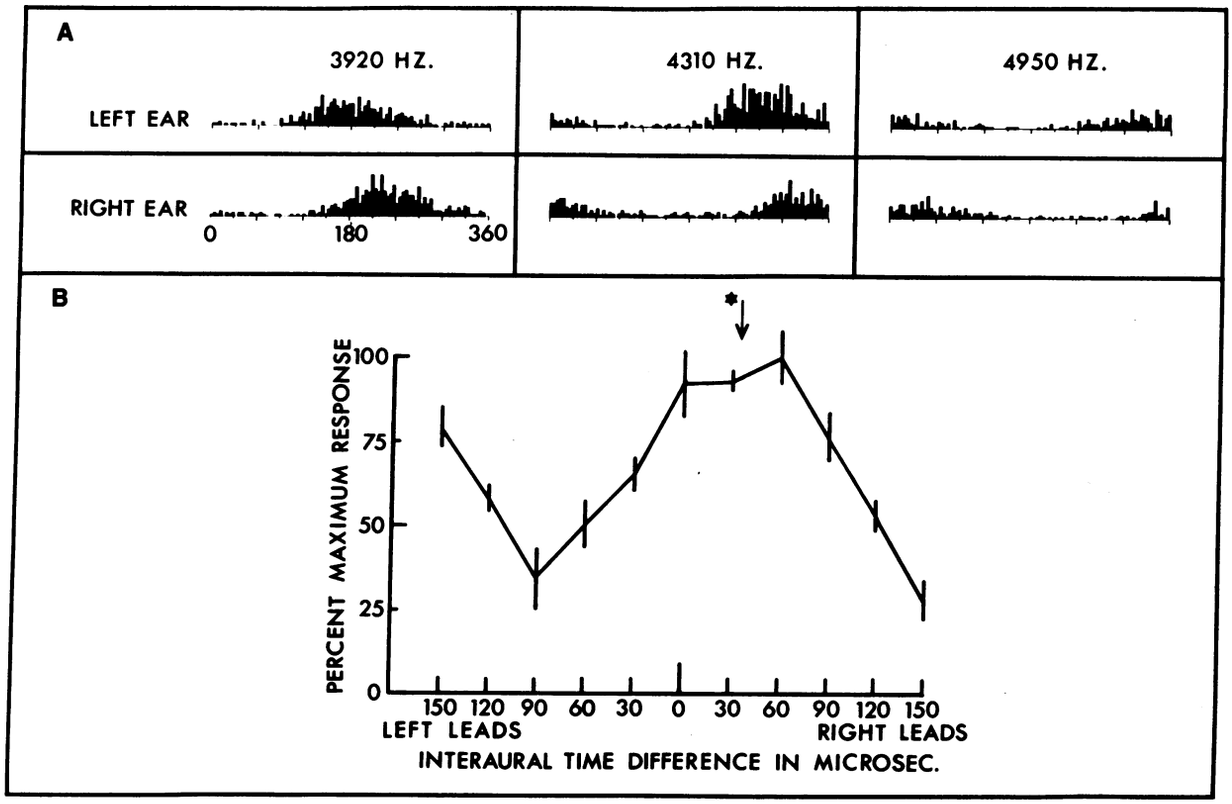

FIG. 1. Response of a laminaris neuron to monaural and binaural tonal stimuli. (*A*) Period histograms of spikes elicited by monaural stimulation for three different frequencies. In all cases, the phase-locked response due to left ear stimulation leads the response due to right ear stimulation by about 40 μsec. (*B*) Normalized response of the same neuron to binaural stimulation as a function of interaural time difference. The response reaches a maximum when the right ear stimulus leads the left by 40 μsec.

imally not only to its characteristic delay but also to interaural time differences that were n tonal periods away from the characteristic delay. In other words, the neuron cannot distinguish dt from $nT + dt$, where dt is interaural time difference, n is an integer, and T is the tonal period. This is because the neuron, using phase-locked inputs, measures time not from a single fixed point such as stimulus onset but from the beginning of each tonal period. Therefore, a laminaris neuron can be said to signal the phase-equivalent of its characteristic delay. For this reason, the present work uses the terms, phase and time, either interchangeably or differently depending on the context in which they occur.

Evoked Potential Recording. Whether laminaris neurons are systematically arranged according to their characteristic delays cannot be precisely determined by extracellular recording from output fibers because of the difficulty in localizing their cell bodies. In addition, mapping studies require reasonably large sample sizes. One of the major reasons for the lack of success in single unit recording within the nucleus is the presence of a large multiunit potential that effectively masks single unit spikes (6–8). However, these evoked potentials provide physiological clues for a map of delays in nucleus laminaris.

In nucleus laminaris, tone bursts elicited a nearly sinusoidal evoked potential containing a major spectral component identical to that of the stimulus tone. This potential, referred to as a neurophonic (9, 10), had 2- to 3-msec latencies and changed in frequency selectivity, amplitude, and phase with recording location. Fig. 2 *A* and *B* shows portions of neurophonics recorded at different depths for ipsilateral and contralateral stimulation. The neurophonic elicited by contralateral and ipsilateral stimulation showed, respectively, a progressive phase advance and delay with increasing depth (relative to the first response of the penetration). The change in the phase of the monaural neurophonics with depth is plotted in Fig. 2*E*. The phase delay due to ipsilateral stimulation began and ended more dorsally than the phase advance due to contralateral stimulation. In both cases, the rapid and systematic phase shifts occurred only within the boundaries of the nucleus.

Binaurally elicited neurophonics were sensitive to variation in recording site and interaural time difference. As mentioned above, the phase of neurophonics differed depending on the side of stimulation. When the phase difference was compensated for by a delay or an advance in the acoustic signal, the binaurally induced neurophonic reached its largest amplitude. This is primarily due to waveform summation, the two monaural responses being brought in phase. The interaural phase difference that elicited a maximal neurophonic amplitude (optimal binaural delay) changed systematically with depth, always shifting toward a greater time lead in the ipsilateral ear (Fig. 2 *C* and *F*). As with monaural neurophonics, site-dependent shifts in optimal binaural delay occurred only within the nucleus. Lesions marking the onset of the systematic shift (Fig. 2*D*, no. 1) were always found on the dorsal edge of the nucleus ($n = 17$), and those marking the end of the phase shift ($n = 20$) were seen exclusively on the ventral edge (Fig. 2*D*, no. 2).

The distribution of optimal binaural delays in the nucleus was determined by systematic sampling of neurophonics in a slab of tissue corresponding to an isofrequency lamina. Each such slab contained a map of optimal binaural delays. The map is best illustrated by isodelay contours (Fig. 3*A*). In each penetration, a lesion was made at the site where optimal delay equaled 15 μsec, ipsilateral ear leading. The lesions at the top of the nucleus (the point in the recordings where systematic changes in optimal binaural delay began) served as reference points for the calculation of isodelay contours. As shown,

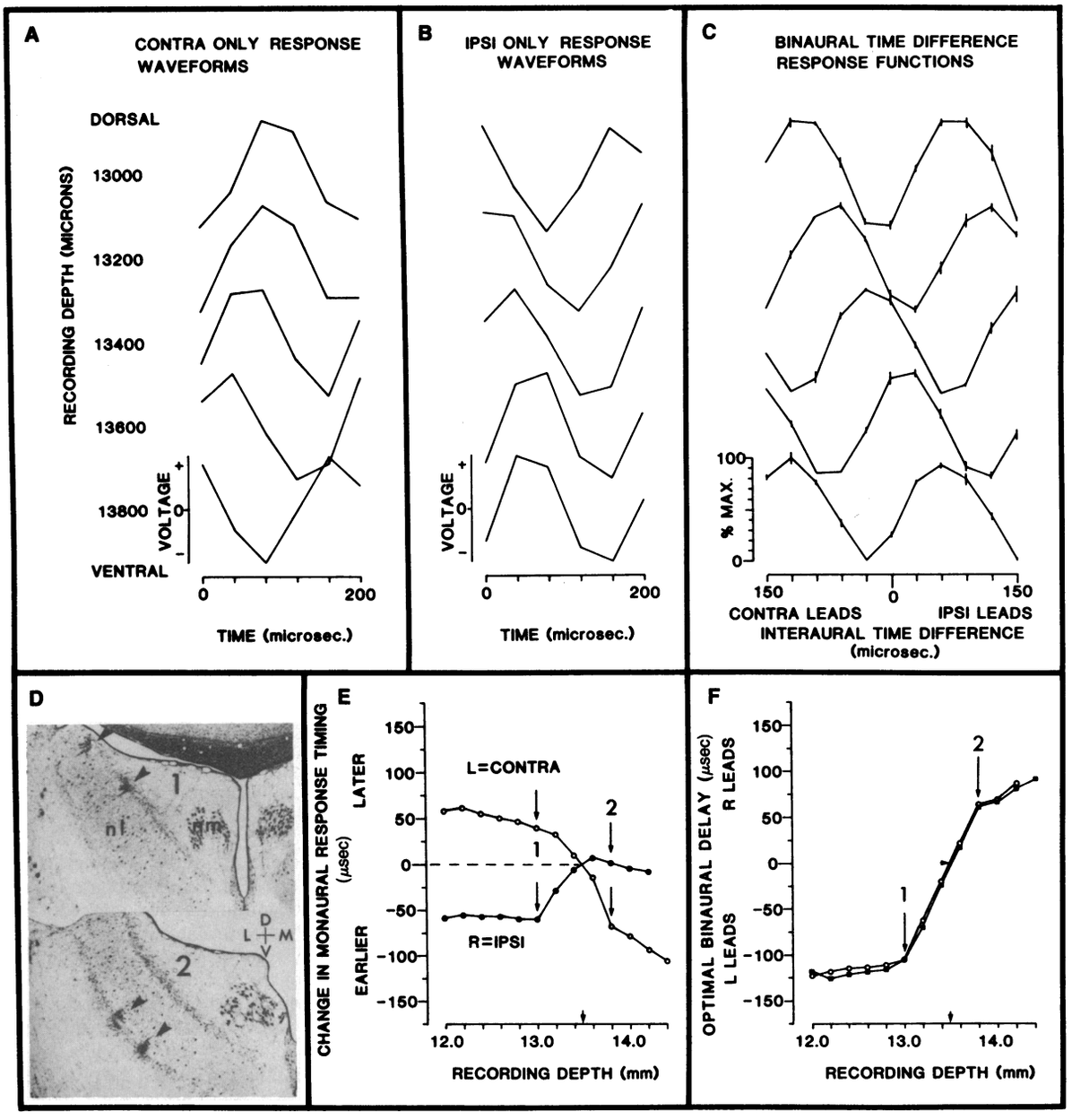

FIG. 2. Site-dependent phase shifts in sinusoidal evoked potentials. The phase of sinusoidal potentials (neurophonics) elicited by a 5.5-kHz tone varies systematically with recording depth, phase advance for contralateral (CONTRA) (*A*) and delay for ipsilateral (IPSI) (*B*) stimulation. The interaural time difference eliciting a peak response in binaural neurophonics shifts toward a greater time lead in the ipsilateral ear with depth (*C*). Changes in optimal binaural delay occur only within the boundaries of the nucleus (*D*). Arrows 1 and 2 indicate the loci where linear shifts in optimal delay began (top) and ended (bottom), respectively. (*E* and *F*) Quantitative analysis of monaural and binaural responses. (*E*) Changes in phase of monaural responses with depth. At each depth, monaural waveforms were cross-correlated with the first monaural waveform recorded in the penetration. Time zero is arbitrarily set to the depth at which the two monaural responses are in phase. (*F*) Change in optimal binaural delay as a function of depth. Squares, optimal binaural delays; circles, time differences between left (L) and right (R) monaural responses determined by cross-correlation of the two. Arrows 1 and 2 in *E* and *F* indicate the criterion for optimal binaural delay shifts for the lesion sites shown in *D*. nm, Nucleus magnocellularis; nl, nucleus laminaris; D, dorsal; V, ventral; L, lateral; M, medial.

isodelay contours run roughly parallel to the dorsal and ventral surfaces of the nucleus. Contralateral delays are found dorsally, whereas ipsilateral delays are found ventrally and medially. Most delays are contralateral, ranging in this case from 130 μsec contralateral to about 50 μsec ipsilateral.

DISCUSSION

Neurons of the mammalian medial superior olivary nucleus (MSO) behave very much like those of nucleus laminaris (4, 11). However, no evidence for delay lines or the place representation of interaural time difference has been reported. Although the ipsilateral–contralateral difference in phase-locking is consistent with a model employing variations in axonal path length, other mechanisms could produce similar behavior in single units. No study of MSO or laminaris has isolated and localized sufficient numbers of single units to demonstrate a map of interaural time difference.

The significance of the evoked potential results depends on how and where the potential is generated. Neurophonics are found only in brain areas where phase-locking occurs, suggesting that the potentials result from summation of

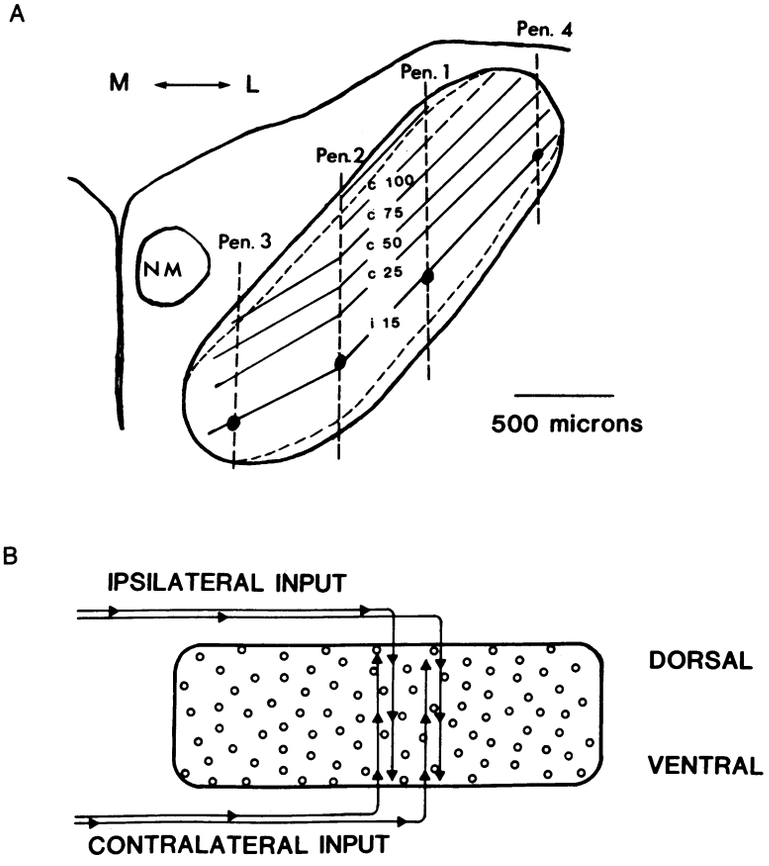

FIG. 3. (*A*) Contour map of optimal binaural delays and model of nucleus laminaris. The map is based on four penetrations (Pen.) in a 5-kHz isofrequency slab. In each penetration, a lesion was made at the site where the optimal binaural delay equaled 15 μsec, ipsilateral ear leading. Lesions are indicated by black dots. Isooptimal delay contours of 100, 75, 50, and 25 μsec (c = contralateral ear leading) and 15 μsec (i = ipsilateral ear leading) are shown. (*B*) Delay-line model of nucleus laminaris. Each isofrequency lamina contains two-dimensional arrays of neurons, which serve as coincidence detectors. Axons from the ipsilateral nucleus magnocellularis (the cochlear nucleus) enter the arrays from their dorsal end, whereas axons from the contralateral nucleus innervate the arrays from their ventral end. The axonal path length to a neuron varies systematically in proportion to the distance from either end. The arrows indicate the direction of signal transmission.

phase-locked spikes of many neurons. Also, the high frequency limits for phase-locking and neurophonics are similar, 3–4 kHz in the cat and 8.5 kHz in the owl (7–13). The site-dependent shifts in selectivity for frequency and phase indicate the local source of neurophonics. The shifts that occurred during a penetration cannot be accounted for by changes in the filtering properties of the recording system. Furthermore, within an isofrequency lamina, neurophonic phase shifts occurred only between the dorsal and the ventral surfaces of the nucleus. Therefore, the cause for the delays must be present between the two surfaces. Incoming axons from nucleus magnocellularis and laminaris neurons show phase locking, implying a tight temporal coupling between input and output spikes. Thus, either or both can contribute to the neurophonic. Whichever the case, we assume that neurophonic phase shifts indicate variations in transmission time.

The bilateral innervation of nucleus laminaris by axons from nucleus magnocellularis suggests that the variation in transmission time is due to differences in axonal paths (14). Axons from the ipsilateral magnocellular nucleus run along the dorsal surface of nucleus laminaris and give off collaterals within their own isofrequency lamina (15). Many of these collaterals course along more or less straight trajectories toward the ventral surface of the nucleus. Axons from the contralateral magnocellular nucleus project collaterals into nucleus laminaris from its ventral surface. Collaterals from the two sides interdigitate as they course through the nucleus in opposite directions, and contacts between the collaterals and the evenly spaced laminaris neurons are either made *en passant* or by short terminal branches. Laminaris neurons have rudimentary dendrites and large somatic spines. It is thus unlikely that transmission delays occur either in the dendrite or somata.

Therefore, it is reasonable to attribute the gradients of neurophonic delays to the trajectories of axon collaterals. As the electrode moves ventrally, it moves closer to the entry point of the contralateral collaterals and farther from that of the ipsilateral fibers. Thus, the delay from the contralateral side decreases and that from the ipsilateral side increases with depth. If the above assumption is correct, the neurophonic delays at a particular locus in the nucleus would indicate the transmission delays from each side to a neuron at that site. By acting as coincidence detectors, laminaris neurons transform the observed map of transmission delay differences to a map of tuning to interaural time difference. A model incorporating these elements of axonal delay lines and coincidence detectors is shown in Fig. 3*B*. The change in neurophonic phase with depth amounts to an average rate of signal transmission of 10.4 m/sec (n = 31, SD = 1.78). The distance over which linear shifts in binaural optimal delay occurred ranged from 600 to 1000 μm (mean, 770 μm). Using these values, we predict an average optimal binaural delay range of about 148 μsec. This is reasonable because the largest behaviorally relevant interaural time difference is ≈170 μsec (2).

Barn owls use interaural time difference for localization in the azimuthal plane. Interaural time difference also defines

the azimuthal axis of the auditory space map in the owl's inferior colliculus (2). Because space is not represented in the cochlea, an auditory space map must be derived by neural processing. Nucleus laminaris is the first site at which neuronal selectivity for, and mapping of, interaural phase difference occurs. Further processing of interaural phase difference involves the sharpening of selectivity, the development of mechanisms to distinguish dt from $dt + nT$, and the topographical projection of the map to higher-order stations, including the auditory space map in the inferior colliculus.

We thank Drs. E. I. Knudsen and T. Takahashi for critically reading the manuscript. The work was supported by National Institutes of Health Grant NS 14617 and a Del E. Webb Postdoctoral Fellowship to W.E.S.

1. Jeffress, L. A. (1948) *J. Comp. Physiol. Psychol.* **41,** 35–39.
2. Moiseff, A. & Konishi, M. (1981) *J. Neurosci.* **1,** 40–48.
3. Moiseff, A. & Konishi, M. (1983) *J. Neurosci.* **3,** 2553–2562.
4. Goldberg, J. M. & Brown, P. B. (1969) *J. Neurophysiol.* **32,** 613–636.
5. Rose, J. E., Gross, N. B., Geisler, C. D. & Hind, J. E. (1966) *J. Neurophysiol.* **29,** 288–314.
6. Tsuchitani, C. & Boudreau, J. C. (1964) *J. Neurophysiol.* **27,** 814–827.
7. Boudreau, J. C. (1965) *Nature (London)* **208,** 1237–1238.
8. Wernick, J. S. & Starr, A. (1968) *J. Neurophysiol.* **31,** 428–441.
9. Weinberger, N. M., Kitzes, L. M. & Goodman, D. A. (1970) *Experientia* **26,** 46–48.
10. Snyder, R. L. & Schreiner, C. E. (1984) *Hearing Res.* **15,** 261–280.
11. Chan, J. C. K. & Yin, T. C. T. (1984) *Soc. Neurosci. Abstr.* **10,** 844.
12. Rose, J. E., Brugge, J. F., Anderson, D. J. & Hind, J. E. (1967) *J. Neurophysiol.* **30,** 769–793.
13. Sullivan, W. E. & Konishi, M. (1984) *J. Neurosci.* **4,** 1787–1799.
14. Carr, C. E., Brecha, N. & Konishi, M. (1985) *Soc. Neurosci. Abstr.* **11,** 735.
15. Parks, T. N. & Rubel, E. W. (1975) *J. Comp. Neurol.* **164,** 435–448.