TIME AND INTENSITY CUES ARE PROCESSED INDEPENDENTLY IN THE AUDITORY SYSTEM OF THE OWL[1]

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Abstract

Space-specific neurons, found in the barn owl's inferior colliculus, respond selectively to a narrow range of interaural time and intensity differences. We show that injecting a local anesthetic into one cochlear nucleus, nucleus magnocellularis, alters the space-specific cell's selectivity for interaural time difference, leaving its selectivity for interaural intensity difference intact. Anesthetizing the other cochlear nucleus, nucleus angularis, has the converse effects. We show also that the space-specific neuron's selectivity for one interaural cue is the same for all effective values of the other cue. We conclude that time and intensity cues are processed in separate neural channels of the barn owl's auditory system and that the two cues operate independently.

The barn owl uses differences in the timing and intensity of a sound at its ears to determine, respectively, the azimuth and elevation of the source (Knudsen and Konishi, 1979). Space-specific neurons (formerly termed "limited-field" or "space-mapped" cells) found in its inferior colliculus are highly selective for these sound localization cues. They are excited only by sounds emanating from a circumscribed region of space or by dichotic stimuli having a particular combination of interaural intensity difference (IID) and interaural time difference (ITD) (Moiseff and Konishi, 1981).

Recent evidence suggests that the auditory pathways leading to the barn owl's inferior colliculus process time and intensity cues independently. The cochlear nuclei, nucleus angularis and nucleus magnocellularis, are specialized to encode either the intensity or the phase of a sound (Sullivan and Konishi, 1984), and the binaural nuclei innervated by them are sensitive to either IID or ITD (Moiseff and Konishi, 1983). We proved that time and intensity are independently processed by monitoring the activity of single space-specific neurons while reducing neural activity in a cochlear nucleus with a local anesthetic. We also demonstrated that under normal, physiological conditions, the processing of one cue does not interfere with that of the other.

Materials and Methods

Our standard experimental procedures, i.e., general anesthesia, surgery, and signal processing, are identical to those already described by Moiseff and Konishi (1981, 1983).

Our procedure for reducing the activity in small volumes of brain tissue is similar to that described by Malpeli and Schiller (1979). A glass micropipette (tip inner diameter = 10 μm) filled with a 1:1 mixture of 5% lidocaine (Xylocaine HCl with glucose, Astra Pharmaceuticals) and 1 M sodium acetate was coupled to a 10-μl Hamilton syringe and inserted into one of the two cochlear nuclei. Multiunit responses to sound, which could be recorded through the pipette, facilitated accurate placement.

While the pipette was in the cochlear nucleus, a single, space-specific unit was isolated with a platinum-iridium microelectrode. First, the unit's selectivity for IID and ITD was assessed by counting the spikes (spike count) it discharged to dichotic stimuli having various ITDs and IIDs. When testing the selectivity of the cell to one cue, we used the most effective value of the other cue, and, when presenting various IIDs, we held the average binaural intensity constant. Between 10 and 300 nl of the lidocaine-sodium acetate mixture (hereafter referred to as "lidocaine") were then injected into the cochlear nucleus, and the tuning of the space-specific unit to interaural cues was monitored. We were able to hold single space-specific units for over 90 min, during which repeated injections into the cochlear nuclei were possible.

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An injection of 250 nl of lidocaine halted the multiunit activity of cells within a 350- to 500-μm radius from the pipette tip for about 15 min. We determined the maximum effective radius (350 to 500 μm) by retracting or advancing the recording pipette and noting the distance at which the amplitude of the multiunit activity matched that of the trace stored in computer memory prior to lidocaine injection. The injections left behind lesions which allowed histological confirmation of the injection site.

Auditory stimuli consisted of 100-msec bursts (5-msec rise/fall time) of noise (7000 Hz center frequency, 15 dB octave roll-off) delivered through dynamic earphones installed in the owls' external ear canals. A digital delay circuit shifted the transient and ongoing time components (Moiseff and Konishi, 1981) of the sound in one ear, and digital attenuators allowed the sound intensity in each ear to be controlled independently.

Definition of terms

Interaural time difference is the difference in the timing of the transient and ongoing components of the sound in one ear with respect to those of the sound in the other ear. Although both components were delayed in this study, space-specific units are known to be selective only for the ongoing component (Moiseff and Konishi, 1981). Therefore, the term "interaural time difference" may be substituted with the term "ongoing time difference" used in earlier reports.

Interaural intensity difference is the difference in root mean square noise levels in the left and right ears.

Average binaural intensity is the sum of the decibel levels in the ears divided by two.

Results

Space-specific neurons are sharply tuned to a particular ITD and IID. For instance, the cell shown in Figure 1 responded maximally when the sound in the right ear was 12 dB louder (IID = 12 dB R>L) and led by 60 μsec (ITD = 60 μsec right ear leading) (Fig. 1, *bottom* and *top rows, pre-inj.*). If ITD or IID deviated from the optimum value by 15 μsec or by 6 dB, respectively, the spike count was halved (widths at half-maximal response = 30 μsec and 12 dB, respectively).

We held a single space-specific unit while anesthetizing nucleus angularis in eight cases (in three birds). Figure 1 shows the effects of anesthetizing the right nucleus angularis (Fig. 2A) on a space-specific neuron of the ipsilateral inferior colliculus (n = 6). Immediately after lidocaine was injected (200 nl) (Fig. 1, *bottom row*, t = 0 min), the spike count versus IID function widened and shifted to the right, the side of the anesthetized nucleus. That is, the cell became maximally responsive to a different IID (IID = 22 dB R>L) and was less selective (half-maximal width = 23 dB). Over the next 15 min, the cell regained its sharp tuning to the original IID (t = 6 to 15 min). The cell remained sharply tuned to the original ITD throughout (Fig. 1, *top row*). In three
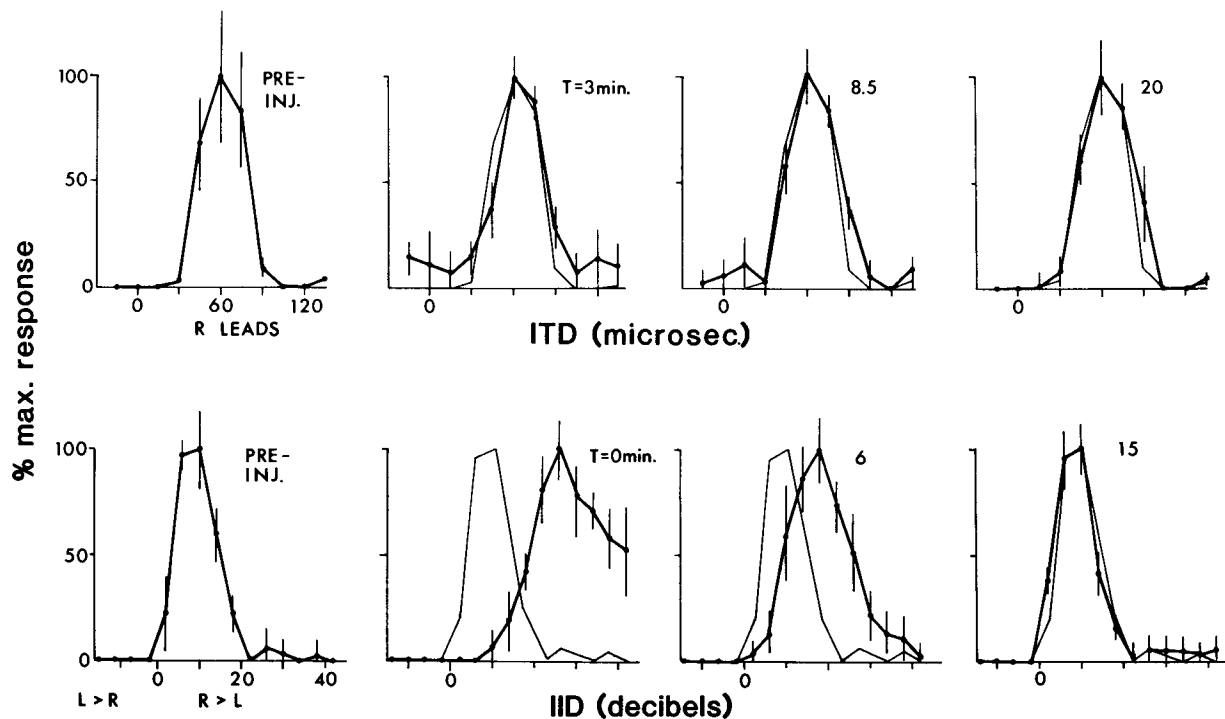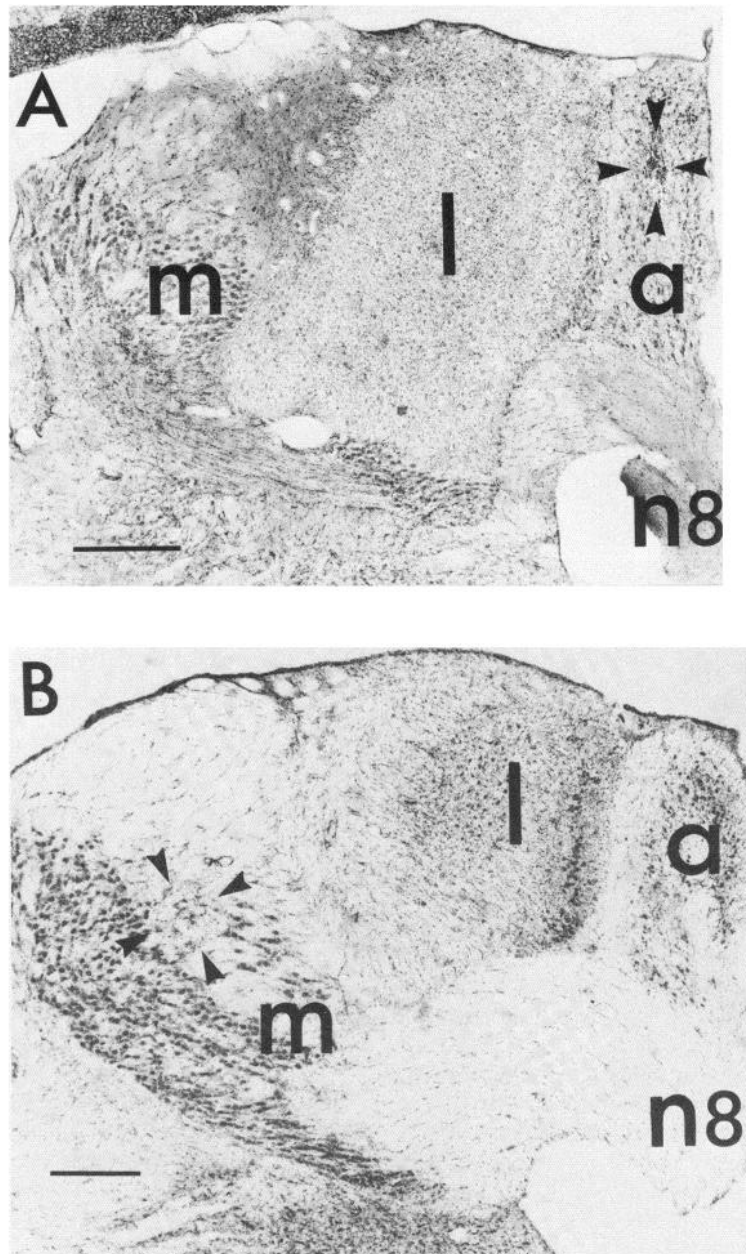

Figure 1. The effect of anesthetizing nucleus angularis on a space-specific cell's (cell 5, owl 141) selectivity to ITD (*top row*) and IID (*bottom row*). The minutes lapsed since the injection of lidocaine (t = 0) are shown at the *upper right* of each plot. The data points of each plot represent the average number of spikes per stimulus presentation (five repetitions) normalized to the maximum spike count. *Vertical bars* represent the standard deviation. The curve obtained prior to lidocaine injection is superimposed on each plot to facilitate comparison. The maximum number of spikes per stimulus presentation for each ITD plot is as follows: PRE-INJ., 6.4; t = 3, 5.4; t = 8.5, 6.6; and t = 20, 7.2. For each IID plot, the maximum number of spikes per stimulus presentation is as follows: PRE-INJ., 6.4; t = 0, 6.2; t = 6, 7.4; and t = 15, 7.4. R and L refer to the sound in right and left ears, respectively.

cases, we reinjected lidocaine after recovery, and, in all of these cases, the above results were reproduced during each anesthetization/recovery cycle. We observed results identical to those above when a space-specific cell of the contralateral inferior colliculus ($n = 2$) was monitored. Specifically, the IID curve broadened, shifted to the side of the anesthetized nucleus angularis, and gradually recovered.

Nucleus laminaris and the auditory (eighth) nerve are close to nucleus angularis at all anteroposterior levels.

Injections of lidocaine into these adjacent auditory structures serve as controls for the site specificity of lidocaine effects. An injection into the eighth nerve alters a space-specific neuron's selectivity for both IID and ITD. A nucleus laminaris injection, which has to be large (1000 nl) to have an effect, causes the ITD curve to shift, without broadening, and has no effect on IID. Thus, ITD is affected by injections of either nucleus laminaris or the eighth nerve, and the effect exclusively on IID results only from injections into nucleus angularis.

Figure 2. Photomicrographs of cresyl violet-stained coronal sections through the brainstems of owl 141 (*A*) and owl 153 (*B*), the birds used for the experiments illustrated by Figures 1 and 3, respectively. Dorsal is up, and lateral is to the right. *Arrowheads* delimit lesions produced by the micropipette used to record multiunit activity and to inject lidocaine. *m*, nucleus magnocellularis; *l*, nucleus laminaris; *a*, nucleus angularis; *n8*, eighth nerve. The *scale bar* at the *lower left* of each photograph denotes 500 μm.
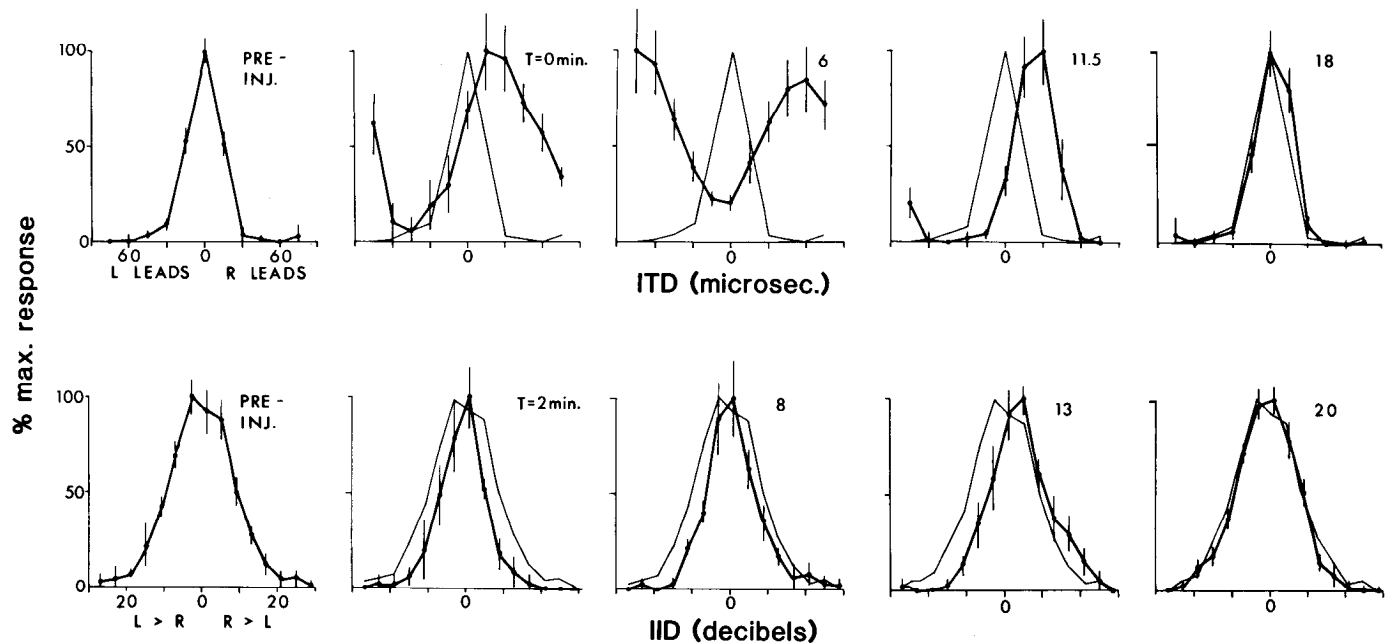
Figure 3. Effects of anesthetizing nucleus magnocellularis on a space-specific cell's (cell 5, owl 153) selectivity to ITD (*top row*) and IID (*bottom row*). All abbreviations and conventions are identical to those of Figure 1. The maximum number of spikes per stimulus presentation for each ITD curve is as follows: PRE-INJ., 23, $t = 0$, 10; $t = 6$, 12; $t = 11.5$, 17; and $t = 18$, 23. The maximum number of spikes per stimulus presentation for each IID plot is as follows: PRE-INJ., 23; $t = 2$, 10; $t = 8$, 13; $t = 13$, 12; and $t = 20$, 25.

We held a single unit while anesthetizing nucleus magnocellularis in nine cases (in four birds). Figure 3 shows the effects of anesthetizing the right nucleus magnocellularis (Fig. 2B) on a space-specific cell of the ipsilateral inferior colliculus ($n = 4$). As can be seen in Figure 2B, nucleus laminaris and the eighth nerve lie far from the injection center, well beyond the 500-μm maximum effective radius. Identical results were obtained from space-specific cells of the contralateral inferior colliculus ($n = 5$). Before lidocaine injection, the cell responded maximally when the stimulus was presented to the ears simultaneously (ITD $= 0$ μsec) (Fig. 3, *top row, pre-inj.*). Its spike count was halved if the sound in either ear led by 15 μsec (half-maximal width $= 30$ μsec). Upon injecting lidocaine (200 nl) ($t = 0$ min), the spike count versus ITD curve broadened and shifted to the right, the side of the anesthetized nucleus magnocellularis. That is, the most effective ITD changed to 15 μsec (right ear leading), and the cell became less selective (half-maximal width $= 72$ μsec). The cell also became responsive to 75 μsec (left ear leading), an ITD that was originally ineffective. Six minutes later, a peak at 75 μsec (left ear leading) and a peak at 60 μsec (right ear leading) were observed ($t = 6$ min). This space-specific neuron responded best to tonal stimuli having periods between 125 μsec (8 kHz) and 142 μsec (7 kHz). Thus, the 135 μsec which separate the two peaks fall within the period range of the frequencies that drive the unit maximally. Over the next 12 min, the cell first became less responsive at 75 μsec (left ear leading), then regained its selectivity

to the original ITD (Fig. 3, *top row*, $t = 11.5$ to 18 min). Except for a slight sharpening,[4] the spike count versus ITD curve remained unchanged (Fig. 3, *bottom row*). In three cases, we reinjected lidocaine after recovery, and in each of these cases, the above results were reproduced.

The results from the lidocaine experiments indicate that time and intensity are processed independently by the pathways leading to the space-specific neuron. Therefore, the selectivity of a space-specific neuron to one cue under normal, physiological circumstances should not be influenced by the value of the other cue, as long as the other cue is effective. This is shown in Figure 4. The *center graphs* of the *top* and *bottom rows*, respectively, show the spike count versus ITD function obtained with the optimal IID and the spike count versus IID function obtained with the optimal ITD. The same functions obtained under nonoptimal conditions are shown in the *flanking graphs*. The sharp tuning of the cell to ITD is unchanged when the IID is 8 dB greater or less than the optimal value (Fig. 4, *top row*; 8 dB L>R, 8 dB R>L). Similarly, its selectivity to IID remains when the ITD is 15 μsec greater or less than the optimal value

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[4] This effect is probably a byproduct of our method of measurement. When spike counts for IID curves are obtained, the optimal ITD value taken from the most recent curve is used. As nucleus magnocellularis recovers from anesthesia, however, the most effective ITD changes (Fig. 2, *top row*). Over the 2.5 min required to take data for an IID plot, the ITD setting used to obtain spike counts deviates further from the optimal value. Consequently, the spike rate is further depressed with each successive IID presented, and the resulting curve is narrower.
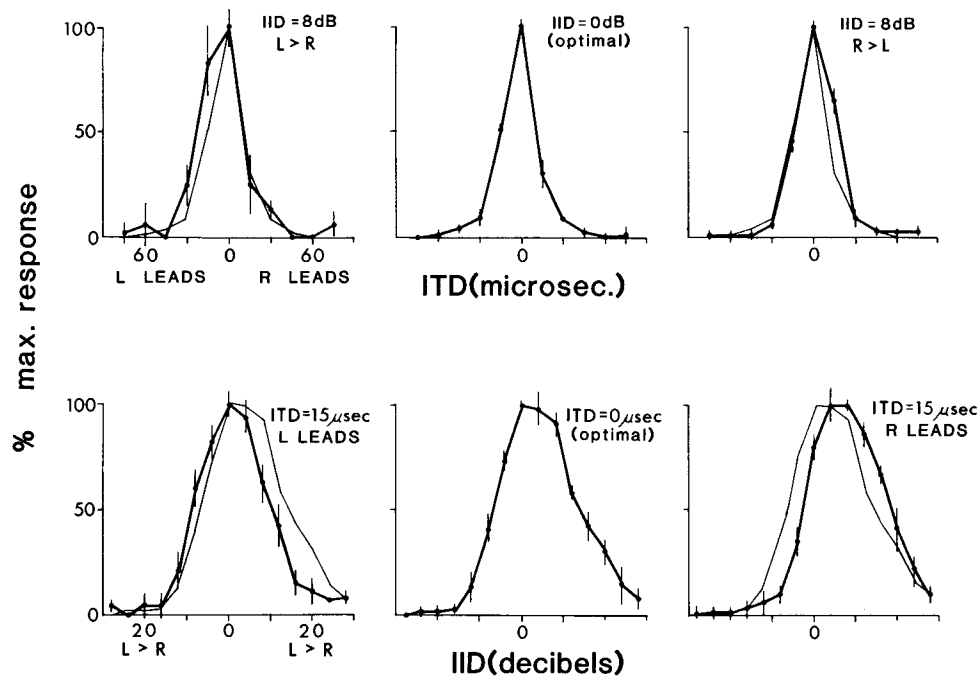
Figure 4. The tuning of a space-specific neuron (cell 11, owl 152) to one interaural cue is not influenced by the value of the other cue. The IID or ITD setting used to obtain data for each plot is at the *upper right*. The curves obtained with optimal IID or ITD values are in the center of each *row* ("optimal") and also superimposed on adjacent graphs to facilitate comparison. Curves obtained with nonoptimal IID or ITD values are shown in the adjacent plots. All other conventions and abbreviations are identical to those of Figure 1.

(Fig. 4, *bottom row*; 15 μsec, left ear leading; 15 μsec, right ear leading).

Discussion

We have demonstrated that a space-specific neuron's selectivity for interaural time and intensity differences is endowed by independent neural channels, involving nucleus magnocellularis and nucleus angularis, respectively. Furthermore, we have shown a physiological consequence of this independence, i.e., the lack of interference in the processing of the two interaural cues.

We consistently observed three effects regardless of which cochlear nucleus was anesthetized. One effect was a broadening of IID and ITD curves. Space-specific neurons are normally inhibited by nonoptimal IID and ITD values (Moiseff and Konishi, 1983) and by sounds originating from immediately outside of their receptive fields (Knudsen and Konishi, 1978). Anesthetizing the cochlear nucleus may decrease this inhibition, causing the cell to lose its high selectivity. Similarly, removal of inhibition may explain the extra peak (at 75 μsec left ear leading) in the ITD curve which appeared when nucleus magnocellularis was anesthetized. It is also possible that this space-specific neuron was normally responsive at 0 μsec and at 135 μsec (left ear leading). The peak at 135 μsec is outside of the ITD range for which the owl had highest acuity and, thus, outside of our testing range. It may simply have been brought into view by the shift in the ITD curve by some 60 μsec to the right (Fig. 3, *top row*, *t* = 6 min). The presence of the peak at 135 μsec, under normal conditions, would be presumed to be due to the

selectivity of the neuron for the interaural phase of the frequency component (7 to 8 kHz, 142- to 125-μsec periods) in the noise to which this cell is most responsive. This preferred interaural phase occurs when the ITD equals an integral multiple of the best frequency's period. This phenomenon has been thoroughly documented for units in the inferior colliculus of the cat (Geisler et al., 1969).

Another effect of anesthetizing the cochlear nuclei was the shifting of tuning curves to the side of the affected nucleus. An explanation is that the diminished activity in the anesthetized nucleus is equivalent to a decrease in the intensity (when nucleus angularis is anesthetized) or an increase in the latency (when nucleus magnocellularis is anesthetized) of the sound on the affected side. Increasing the intensity or lead time offsets these effects, and the maximum response occurs at these new values of IID and ITD. Implicit in this explanation are the assumptions that space-specific neurons are ultimately selective for the difference in the amount of activity (i.e., spike rate per cell multiplied by the number of active cells) in the angular nuclei and for the difference in the timing of the activity in the magnocellular nuclei. These assumptions are reasonable because Sullivan and Konishi (1984) have shown that sound intensity is effectively represented by the spike rate of nucleus angularis neurons and that the cells of nucleus magnocellularis accurately preserve the timing of sounds by phase locking their discharge to the stimulus waveform.

The final effect was that space-specific neurons regained their selectivity to IID and ITD, not in an all-or-none fashion but gradually. As lidocaine wears off, the

number of excitable cells and/or the spike rate of each cell increases. The recovery of the tuning curves of the space-specific neurons may reflect the gradual restoration of these quantities.

The segregation of the two channels reflects the specialization of the barn owl's peripheral auditory apparatus. Its ears are not only separated but directed such that the right and left ears are more sensitive to sound originating from above and below the horizon, respectively (Payne, 1971). This vertical disparity introduces an interaural intensity difference, the magnitude of which varies with the elevation of the source. The horizontal separation of the ears introduces a difference in the interaural timing of the sound, and the magnitude of this difference depends on the azimuth of the source. Time differences of noise stimuli are largely unaffected by stimulus elevation, and intensity differences are largely unaffected by stimulus azimuth (A. Moiseff and M. Konishi, unpublished data). Time and intensity differences, therefore, vary independently of one another, and it is critical to the barn owl that the two cues not interfere with each other; that, for example, a louder sound in one ear not be interpreted as having been heard earlier in that ear. Devoting a separate channel to each interaural cue is an effective, and perhaps the simplest, way to prevent such confusion. It may be a general rule of CNS organization that behaviorally relevant stimulus parameters are segregated and processed in separate neural pathways.

Preliminary anatomical evidence suggests that parallel pathways from the cochlear nuclei to the inferior colliculus exist. The path from nucleus magnocellularis to the inferior colliculus involves, in ascending order, nucleus laminaris and nucleus ventralis lemnisci lateralis (VLV) pars anterior. The cells of the nuclei in this pathway are sensitive to ITD but not to IID (Moiseff and Konishi, 1983). The pathway from nucleus angularis reaches the inferior colliculus via VLV pars posterior in which cells are sensitive to IID but not to ITD (Moiseff and Konishi, 1983). Direct projections to the inferior colliculus from nucleus angularis and nucleus laminaris have also been observed. These parallel pathways may be the anatomical substrates of the independent time and intensity channels.

Single unit studies have shown that there are cells which respond exclusively to IID or ITD in the mammalian superior olivary complex (Boudreau and Tsuchitani, 1968; Goldberg and Brown, 1969) and inferior colliculus (Rose et al., 1966; Geisler et al., 1969). The existence of distinct time and intensity channels in the mammalian auditory system has been suggested, largely on this basis (Knudsen, 1983). The segregation of time

and intensity in mammals, however, occurs according to frequency. It is generally assumed, based on psychophysical data, that mammals use IID and ITD for determining the azimuths of high and low frequencies, respectively. Thus, in mammals, the two channels represent redundant mechanisms for sound localization in a single spatial dimension, i.e., azimuth. By contrast, in the owl, neural processing of time and intensity operates in the same frequency range (4 to 8 kHz) and, along with its peripheral specializations, allows the owl to perceive an additional dimension, i.e., elevation. Therefore, the separation of time and intensity codes is a design feature that reflects this unique, behavioral capability of the barn owl.

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