the concept that small enzyme reactors may provide a rapid, selective means for removing toxic substances from blood (*16, 18*).

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Vision Guides the Adjustment of Auditory Localization in Young Barn Owls

Abstract. *Barn owls raised with one ear plugged make systematic errors in auditory localization when the earplug is removed. Young owls correct their localization errors within a few weeks. However, such animals did not correct their auditory localization errors when deprived of vision. Moreover, when prisms were mounted in front of their eyes, they adjusted their auditory localization to match the visual error induced by the prisms, as long as the visual and auditory errors were within the same quadrant of directions. The results demonstrate that, during development, the visual system provides the spatial reference for fine-tuning auditory localization.*

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Sensory space does not project directly onto the sensory surface of the ear in the way that it does onto the eye or the body surface. As a consequence, the auditory system must derive spatial information indirectly from a variety of acoustic cues. These spatial cues depend on the size and shape of the head and ears and hence change as the head and ears grow. How then, might an animal maintain accurate sound localization during maturation? Young barn owls adjust their interpretation of auditory spatial cues on the basis of experience (*1*). Owls raised with one ear occluded learn, within 4 to 6 weeks, to localize sounds accurately using the altered auditory cues imposed by the earplug. When the earplug is removed, young owls make large localization errors, which they correct over a period of weeks.

We have investigated the signal that guides the adjustment of auditory localization in maturing barn owls. Of all senses, vision provides the most detailed spatial information to the brain. Even in owls, in which hearing is highly developed and visual acuity is relatively poor, the spatial resolving power of vision is still superior to that of audition (*2*). Moreover, in humans, vision strongly influences the perception of sound source location (*3*). Therefore, we hypothesized that vision plays an important role in adjusting errors in auditory localization in barn owls.

We monaurally occluded nine baby barn owls aged 26 to 44 days (*4*). The animals remained monoaurally occluded for 41 to 97 days (Table 1). During this time they were trained to orient their heads toward auditory (noise burst) and visual (light-emitting diode) stimuli, which were presented at random locations in a darkened, sound-attenuating chamber (*5*). One week before an earplug was removed, we attached spectacle frames to each owl (*6*). The mean of more than 100 responses to visual stimuli with the spectacle frames empty defined the head-centered spatial origin for each owl. During the experiments, the accuracy of auditory and visual localization was computed on the basis of this reference value.

The experiments began on the day the earplug was removed. Immediately after earplug removal, the responses of the owl to auditory and to visual stimuli were measured. Because auditory and visual stimuli were presented independently and in a darkened chamber, visual capture did not influence these responses (*3*). Owls that had been raised with the right ear plugged oriented to the right and above the auditory stimulus, whereas owls that had been raised with the left ear plugged oriented to the left and below the auditory stimulus (*7*). Two animals were allowed normal vision and served as controls; five animals were fitted with Fresnel prisms (*8*), which deviated vision by 10°, and two animals were fitted with opaque occluders which prevented vision totally (Table 1). After 28 days, the opaque occluders were replaced with prisms. Most of the owls were exposed to several different prism orientations, each orientation being maintained for periods of 22 to 68 days. The final experiment in every case was to remove the prisms and follow the recovery of accurate auditory localization.

Owls 1 and 2, which were permitted normal vision, adjusted their auditory errors rapidly (Fig. 1, A and B). The errors of both birds diminished at average rates of 0.7° per day, and after 28 days these birds were localizing sounds with mean errors of less than 3°, our criterion for normal localization accuracy (*1*). In contrast, owls 3 and 4, which had their eyes covered with opaque occluders after the earplugs were removed, maintained constant auditory errors for periods of 28 days (Fig. 1C). Since without vision these animals did not adjust their auditory errors, vision must be essential to trigger the adjustment process, to guide the adjustment, or both.

The role of vision was clarified by the first experiment done on owl 5. As shown in Fig. 1D, the bird had an initial auditory error of right 6.4° and up 8.4°. Prisms were mounted on this owl to

Table 1. Auditory and visual histories of all owls studied. Abbreviations: L, left; R, right; U, up; D, down.

Owl	Auditory history			Visual history (sequence of visual experiments)
	Ear occluded	Age (days) at which earplug was		
		Inserted	Removed	
1	L	27	113	Control
2	R	35	76	Control
3	L	31	75	Occluders; prisms: LU, RU, off
4	R	36	85	Occluders; prisms: R, RU, RD, off
5	R	43	92	Prisms: RU, U, off, L, D, off
6	R	28	94	Prisms: LD, LU, U, off
7	R	44	141	Prisms: R, off
8	R	26	75	Prisms: LU, L, off
9	L	28	78	Prisms: LU, LD, off

deviate its visual world in the same direction (the actual prism setting was right 5.4° and up 8.4°). After 29 days, the owl's auditory error was essentially unchanged at right 5.4° and up 7.0°. Thus, when vision is allowed, but there is no mismatch between visual and auditory space, no adjustment of sound localization occurs.

We investigated the extent to which vision controls the adjustment process by establishing various mismatches between visual and auditory space. It became apparent immediately that a visual-auditory mismatch could induce the selective adjustment of either the horizontal or the vertical component of an auditory localization error (Fig. 2). Moreover, in 12 of 12 experiments, the owls adjusted their auditory localization to match the visual error induced by the prisms, as long as the visual error was in the same quadrant of directions as the initial auditory error. For example, owls with initial auditory errors to the right and up adjusted their auditory errors to match prism-induced visual errors of straight-right (two experiments), straight-up (two experiments), various directions to the right and up (two experiments), or back to 0°, 0° (six experiments).

In contrast, whenever either the horizontal or the vertical component of the prism-induced visual error was opposite in direction to the initial auditory error, the owl either adjusted only partially (four experiments), or failed to make any systematic adjustment at all (four experiments). For example, owl 9 experienced auditory and visual errors that were in opposite vertical directions (Fig. 2B): its initial auditory error was left 7.8° and down 8.5° and the visual error induced by the prisms was left 5.2° and up 8.4°. Although this owl adjusted the vertical component of its auditory error by 9.3° in just 17 days, wearing the prisms an additional 28 days did not cause a significant upward error. The auditory error never went more than 2.1° into the upward direction. A similar result is shown for owl 4 (Fig. 2C). However, in this older bird, the adjustment was slower and even less complete. Owl 8 had auditory and visual errors that were in opposite horizontal directions (Fig. 2D): its initial auditory error was right 8.3° and up 11.5°, and its prism-induced visual error was left 5.0° and up 8.7°. After 38 days, the auditory error had changed to left 1.9° and up 6.8°, but during the subsequent 36 days no further adjustment was observed.

The experimental series performed on owl 6 (Fig. 2E) also demonstrated the

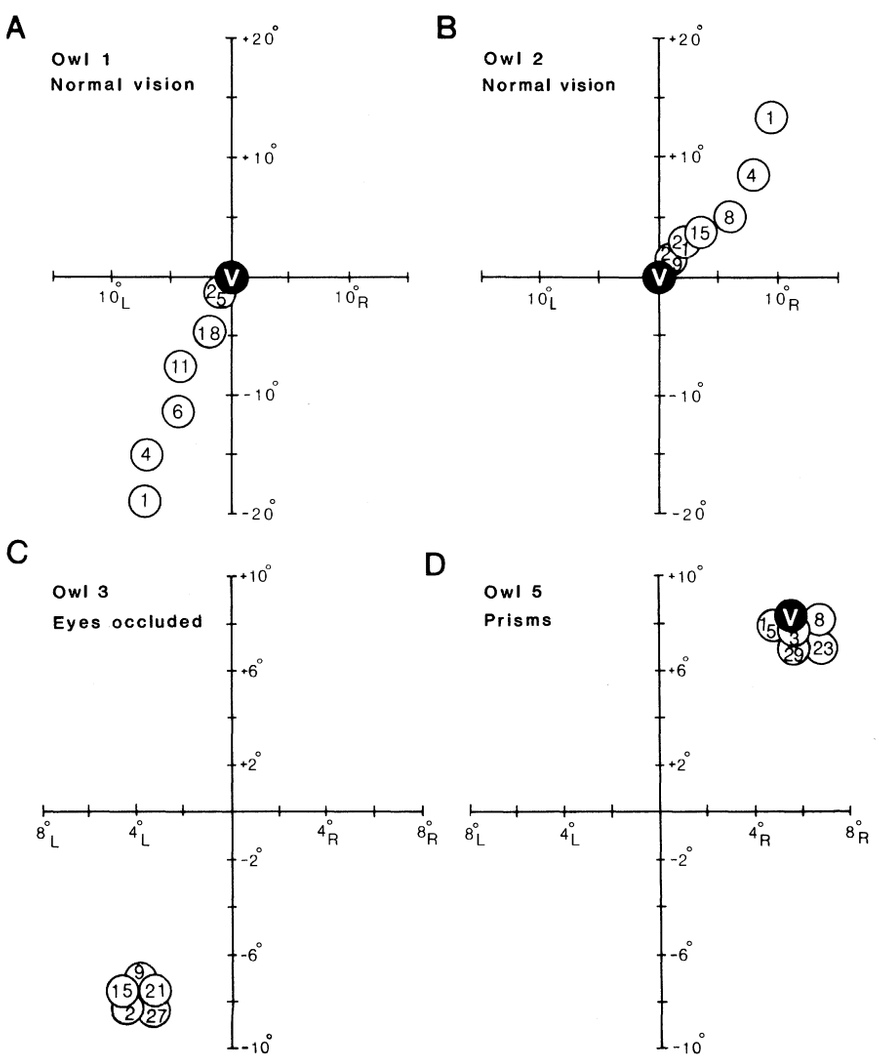

Fig. 1. Auditory localization errors following earplug removal in four barn owls that had been raised with one ear plugged. The origin of each coordinate system was defined by the mean of more than 100 responses by the owl to the visual stimulus measured without opaque occluders or prisms in the spectacle frames. Each open circle represents the mean of 15 to 25 responses of the owl to the auditory stimulus; the number indicates the number of days after earplug removal on which the data were gathered. Standard deviations of the auditory responses ranged from 0.8° to 3.8°, but were typically between 1.0° and 2.5°. The owls were tested every 2 to 3 days, but because the data overlapped extensively, only a few representative points are shown. Circled V's indicate the visual error attributable to the prisms.

limited effectiveness of a visual-auditory mismatch in guiding the adjustment of sound localization when the visual and auditory errors were in opposite directions. In experiment 1, the prisms were oriented so that the visual error (to the left and down) was opposite in both dimensions to the auditory error (to the right and up). Although the discrepancy between auditory and visual localization was large in this case, it was well within the range of adjustment of auditory localization (for example, Fig. 1, A and B). Nevertheless, the owl's auditory error remained essentially unchanged for 26 days. In experiment 2, the prisms were oriented so that the visual and auditory errors had the same vertical components, but the visual error was still leftward and the auditory error rightward. After 23 days, the auditory error was unchanged. However, in experiment 3, the prisms were oriented so that the visual error was 10° straight up, and the owl adjusted its auditory error in just 13 days to right 1.8° and up 8.8°. Finally, the prisms were removed, and the owl corrected the residual vertical component of its auditory error in 9 days. This animal made horizontal and vertical adjustments, but only when the visual and auditory errors were in the same quadrant of directions.

These results are consistent with the hypothesis that a mismatch between visual and auditory space establishes a corrective force that guides a reinterpretation of auditory cues. When vision is prevented, or when visual and auditory space are matched but incorrect, no corrective force is generated and no adjustment in auditory localization occurs. Apparently, spatial information provided by other senses is inadequate to cause such an adjustment. However, the influence of vision had definite limitations in these experiments. Vision altered the magnitude, but not the sign, of the auditory error. This implies that there is, in addition, a nonvisual spatial referent that

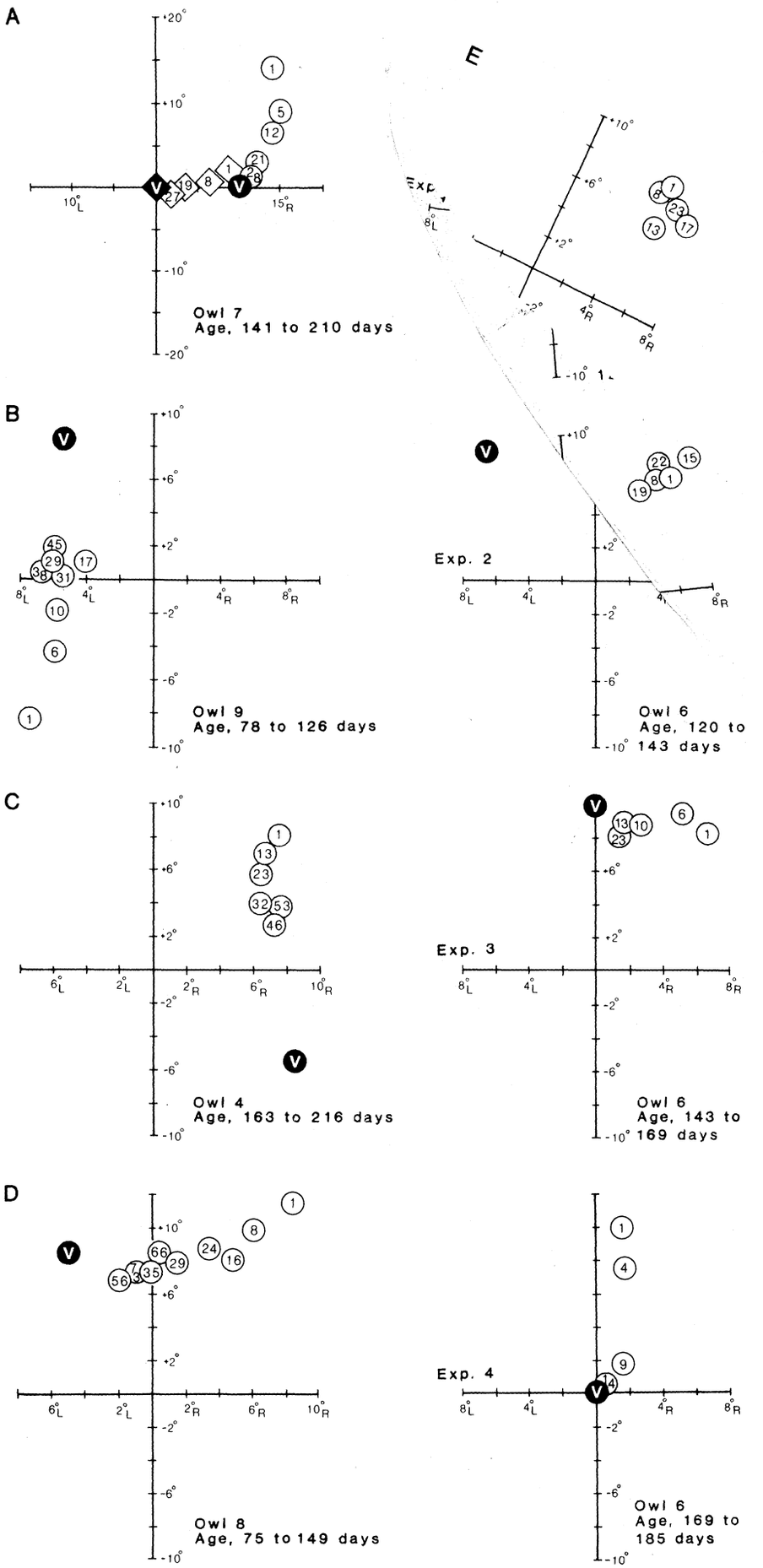

Fig. 2. Adjustments of auditory localization errors by birds wearing prisms. The origin of each coordinate system was based on more than 100 visual responses by the bird before prisms were installed. Numbered circles represent mean auditory errors. The numbers indicate the number of days since the prisms were installed. The circled V's represent the owl's mean visual error caused by the prisms. As in Fig. 1, only a few representative points are shown. In (A), circles represent data from the first experiment on owl 7; the diamonds represent data from the second experiment (prisms off). In (E), data from four sequential experiments on owl 6 are presented in the order in which they were performed.

confines the adjustment process to one quadrant of directions.

The influence of vision on auditory localization apparently decreases with age. Before an owl is 50 to 60 days old, it can adjust its auditory error in any direction, presumably guided by vision (*1*). In the experiments presented here, in which the owls were between 75 and 220 days old, younger birds made larger and more rapid adjustments than did older birds (for example, compare Fig. 2B with 2C), but in no case could an owl be induced to change the sign of its auditory error. Finally, adult owls (more than 7 months old) maintain auditory localization errors indefinitely, even when they experience normal vision (*1*). Thus, the ability of vision to generate a corrective force, or the ability of the auditory system to respond to it, or both, diminish with age. This corrective force exerted by the visual system on the auditory system provides a mechanism for fine-tuning the associations that underlie auditory localization during development.

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5. The method used in this study has been described in detail (*1*). Briefly, we measured auditory localization by comparing the accuracy with which an owl oriented its head to auditory and to visual stimuli. The stimuli were presented from a remotely controlled, movable speaker and light source, which were positioned at a new, random location before every trial. Auditory stimuli consisted of repeated noise bursts presented at 10- to 50-dB sound-pressure level (re 20 μPa). Visual stimuli consisted of a continuous glow from a light-emitting diode. A trial consisted of a presentation of either the auditory or the visual stimulus; the final head orientation (as indicated by an infrared beam reflected from a mirror mounted on the owl's head) relative to the true location of the stimulus was recorded. The stimulus continued until the animal settled on a particular direction. A test session included 15 to 25 responses to auditory stimuli and an equivalent number to visual stimuli. When an owl responded to a stimulus with a quick movement of the head followed by a steady fixation, it was given a food reward. Because the reward was not contingent on the location to which the owl oriented, the reward did not bias the localization response.
6. Owls were anesthetized with halothane and nitrous oxide, and a metal plate was secured to the skull with screws and dental cement. The spectacle frames were bolted to this plate. Each frame was 10 mm in diameter and permitted approximately a 70° field of view as determined by retinoscopy. The frames did not physically obstruct or interfere with the external ears.
7. The auditory localization error was defined as the difference between the owl's mean response to the acoustic stimulus, based on 15 to 25 responses measured that day, and the reference value, which was based on more than 100 visual responses recorded before prisms or occluders were installed. The vertical component of the barn owl's auditory error results from the fact that, due to an asymmetry in its external ears, interaural intensity differences indicate the vertical location of the sound source.
8. The prisms were 20-diopter Fresnel (Optical Sciences Group). They were oriented in the spectacle frames through the use of an optical bench. Their orientation on the animal was determined by comparing the visual responses of the bird before and after mounting the spectacles.

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Evolution of Bunyaviruses by Genome Reassortment in Dually Infected Mosquitoes (*Aedes triseriatus*)

Abstract. Aedes triseriatus *mosquitoes became dually infected after ingesting two mutants of LaCrosse (LAC) virus simultaneously or after ingesting, by interrupted feeding, the two viruses sequentially within a 2-day period. After 2 weeks of incubation, approximately 25 percent of the vectors contained new virus genotypes as the result of RNA segment reassortment. New viruses were transmitted when the mosquitoes fed on mice. Viruses ingested more than 2 days after the initial infecting virus did not cause superinfection of the mosquito vectors.*

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The bunyavirus genome consists of three negative-strand RNA segments, designated L (large), M (medium), and S (small) (*1*). The M RNA segment codes for the two virion glycoproteins (G1 and G2) and a nonstructural protein (NS_M). The S RNA codes for the nucleocapsid protein and a nonstructural protein (NS_S). The L RNA presumably codes for a polymerase (*1*). The segmentation of the genome provides a mechanism for natural evolution of bunyaviruses by reassortment of RNA segments as described for influenza viruses (*2*). Bunyavirus segment reassortment has been reported in vitro, in vivo in mosquitoes, and in nature (*1, 3*). Either the vertebrate host or the vector could be the site for reassortment. Studies to demonstrate bunyavirus genome reassortment in vertebrates have been unsuccessful. In contrast, high-frequency reassortment was detected when mosquitoes (*Aedes triseriatus*) were intrathoracically inoculated with La Crosse (LAC) virus and snowshoe hare (SSH) bunyaviruses (*3*). Thus, it seemed that the vector would be the site of segment reassortment in nature. However, subsequent studies complicated this hypothesis; interference to superinfection was demonstrated between related bunyaviruses in intrathoracically inoculated mosquitoes (*4*). Intrathoracic inoculation is not a natural route of infection. However, if mosquitoes became resistant to superinfection by natural routes, opportunities for dual infection in nature would be limited. Many mosquitoes exhibit a behavior called interrupted feeding, which could preclude interference (*5*). If defensive behavior of the host interrupts the mosquito during engorgement, the vector may finish engorgement on an alternate host. Thus, a mosquito could ingest a blood meal from two different vertebrate hosts viremic with two different viruses in a period of time short enough to preclude interference. A series of experiments was conducted to

Table 1. Phenotype of stock viruses after passage in mosquitoes.

Virus	Meal titer*	33°C		40°C	
		Infection frequency†	Mean titer‡	Infection frequency	Mean titer
LAC wt	7.0–7.3	5/5	1.6×10^5	5/5	1.1×10^5
LAC-II-5§	6.8–7.1	5/5	1.7×10^5	0/5	<10
LAC-I-16	6.5–6.6	5/5	9.9×10^4	0/5	<10

*Log_{10} [tissue culture infective dose $(TCID)_{50}$ per milliliter]. Range of titers of viruses used to infect mosquitoes in three experiments. †Number infected/number tested. ‡Plaque-forming units per mosquito. §The ts mutant viruses have been assigned to complementation-recombination groups. Group II mutants contain a ts lesion in the L RNA segment; group I mutants contain the ts lesion in the M RNA segment (*1*). The permissive temperature is 33°C; the nonpermissive temperature is 40°C.