to two general mechanisms: male dispersal and reluctance of females to copulate with male relatives. Existence of these mechanisms is easily understood if, as is the case with many plants and animals (*23*), inbreeding commonly leads to genetically inferior offspring. I have few data bearing on this issue. Of the two females that copulated with a male relative, one probably never gave birth and offspring of the other were found dead aboveground shortly after weaning. These two cases are inconclusive, since mortality is also high among young of outbred litters (*6*).

If prairie dogs avoid extreme inbreeding, then the frequency of heterozygotes at polymorphic loci should be higher than that expected under conditions of Hardy-Weinberg equilibrium (*24*). At the four polymorphic loci examined, Foltz and I found (*25*) that, as predicted, there was a consistent excess of heterozygotes in 1978, 1979, and 1980.

Behavioral and physiological avoidance of copulation with male relatives in the home coterie (a kind of female choice) is probably an evolved mechanism of outbreeding. Male dispersal patterns may also have evolved primarily to promote outbreeding. However, it is also possible that male dispersal patterns are secondary consequences of female choice (*26*): why should a male remain in a coterie if his female relatives there are unlikely to mate with him?

Numerous investigators have demonstrated one or two mechanisms by which individuals avoid inbreeding (*4*), but single mechanisms of outbreeding usually have alternative explanations (*1, 2*). Alternative explanations become less parsimonious when several different mechanisms all suggest the same conclusion. Four mechanisms are described for prairie dogs; except possibly for humans (*5*), so many mechanisms have not previously been implicated in the maintenance of outbreeding.

Even when individuals avoid mating with close genetic relatives such as parents, offspring, and siblings, inbreeding coefficients can be high if populations are small and isolated or if individuals regularly mate with more distant relatives such as nieces, nephews, and first cousins (*1, 27*). Black-tail colonies are usually large and there is regular immigration of males (*6, 25*) (Fig. 1). Whether individuals avoid mating with their more distant genetic relatives is not yet known.

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9. These behaviors include (i) a mating call by the male, (ii) the estrous female aboveground 10 to 30 minutes after other colony residents have submerged for the night, and (iii) postcopulatory licking of the vulva by the female, postcopulatory licking of the penis by the male, or both. These behaviors are also associated with rare aboveground copulations (*10*). Each female comes into estrus on one day only each year.
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12. In 1978, for example, 16 of 25 (64.0 percent) adult males of known age at the study colony were ≥ 4 years old; in 1979, 5 of 28 (17.9 percent) adult males of known age were ≥ 5 years old.
13. Adult males that disappeared after 2 years in the same breeding coterie may have died rather than dispersed; therefore, I did not use data from these males in Table 1.
14. Since the adult male sires most of the offspring born into his coterie during his residency (*6, 8, 10*), I assumed that the adult male in a female's natal coterie in her year of birth was her father; for all 15 of the 28 cases in Table 2 for which the critical blood samples were available, the samples supported this assumption.
15. Of the other 92 estrous females, 20 (21.7 percent) copulated with a second adult male from a different coterie.
16. This nephew was the only male at the study colony known to breed as a yearling.
17. In cases one through six, the unrelated male chosen by the estrous female was not consistently either older or heavier than the available male relative.
18. The male in case eight moved to an adjacent coterie in his first year and then returned to his natal coterie as a 2 year old; no other male returned to his natal coterie after dispersing as a yearling.
19. Although the female in case eight weaned litters in the two previous years, she showed no sign of pregnancy (*6, 10*) when her 2-year-old son was back in her coterie, indicating that we did not simply fail to detect a subtle estrus.
20. Field assistants and I failed to detect several estrous periods in 1978, our first year of breeding observations; therefore, 1978 data are not included here.
21. This female weighed 860 g on 28 May 1979 when she was lactating and only 418 g on 7 May 1980, just before she disappeared, when she was not lactating.
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Inner Ear: Dye Injection Reveals Peripheral Origins of Specific Sensitivities

Abstract. *In the American bullfrog* (Rana catesbeiana) *tracing of functionally identified, dye-filled fibers of the eighth cranial nerve to their peripheral origins has provided the first precise functional overlays for the microstructural maps of inner-ear sensory surfaces.*

The inner ear of the frog comprises eight sensory surfaces and the various structures accompanying each of them (*1*). Certain features of those structures suggest the general class of sensitivity associated with each surface: the semicircular canal accompanying each of the three cristae implies sensitivity to rotational motion about a particular axis; the calciferous mass accompanying each of the three maculae implies sensitivity to gravity or to linear motion; and the intimate connections between the chambers of the basilar and amphibian papillae and the sound-conducting apparatus of the middle ear imply auditory sensitivity. These implications have been supported by electrophysiological and behavioral experiments of earlier investigators (Table 1).

None of the eight sensory surfaces is topographically uniform. For example, each macula (utricular, saccular, and lagenar) comprises two fields (a central field surrounded by a peripheral field) with distinctly different receptor cells (hair cells) (*2*). On the utricular and lagenar maculae, the central fields are thin

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Table 1. Putative peripheral origins of sensitivities in the bullfrog inner ear as estimated by previous studies.

Sensitivity	Peripheral origin
Auditory	
>1000 Hz	Basilar papilla (*9*)
<1000 Hz	Amphibian papilla and saccular macula (*9, 10*)
Seismic	Amphibian papilla, saccular and lagenar maculae (*11*)
Vestibular	
Gravitational	Utricular, saccular, and lagenar maculae (*12*)
Rotational	Semicircular canal cristae (*12, 13*)

bands stretching nearly from one end of the macular surface to the other and occupying less than 20 percent of the macular area. Over the band in each case is the striola (*3*), a specialized region of the otoconial membrane, a gelatinous structure that overlies the hair cells and bears the calciferous mass. The hair cells within the band are markedly larger than those in the peripheral field, and their surface topographies differ markedly from those of the peripheral hair cells (*2*). On the saccular macula, the central field occupies more than 90 percent of the surface area. The sacculus of the bullfrog lacks a striola, but the hair cells of the central field are larger than those of the peripheral field and their surface topographies differ from those of the peripheral hair cells (*4*).

The hair cells of the basilar papilla exhibit a distinctive mediolateral gradation of surface topographies, with those in the medial two or three rows having ciliary arrays in direct contact with an overlying gelatinous structure, the tectorium, and those in the more lateral rows projecting their ciliary arrays freely into the fluid (endolymph) covering the papillar surface (*5*). The hair cells of the amphibian papilla also show a mediolateral gradation of surface topographies, but all of their ciliary arrays apparently contact the amphibian papillar tectorium (*6*). The thickness of the amphibian papillar tectorium itself is graded with the thickest part of the tectorium being rostral and the thinnest caudal (*6, 7*).

One might presume that these structural variations correspond directly to variations in sensitivity (*2, 8*). Although the methods used in previous experiments (*9–13*) had the physiological resolution required to detect many candidate sensitivity variations, they lacked the anatomical resolution required to map those sensitivity variations over the individual sensory surfaces. In a few cases, resolution even to a given surface has been subject to debate. To obtain the resolution necessary to generate sensitivity maps over the individual sensory surfaces, we used glass microelectrodes filled with the anionic fluorescent dye Lucifer yellow (*14*) to identify and mark individual afferent axons in the eighth cranial nerve of the bullfrog (*15*). Penetration of individual axons was accomplished in the cranial cavity, and the bony capsule of the ear was left unopened, with intact circulation. Once an axon was penetrated, its sensitivity was identified through application of auditory, seismic, and vestibular stimuli (*16*), after which the dye was injected electrophoretically.

After fixation and clearing, the entire eighth nerve and inner ear were placed under a fluorescence microscope, and the dye-filled axon was traced to its peripheral origin. Autofluorescence of the hair cells allowed us to determine the location of the peripheral arborization of the axon within the macula or papilla and, often, to identify the set of hair cells innervated by the axon (Fig. 1). For example, within the three maculae, the autofluorescing hair cells of the central fields were easily distinguishable from those of the peripheral fields on the basis of size. The location of a terminal arborization within a given field was determined by counting the rows of hair cells from the edge of the field to the site of the arborization. To date, a few more than 100 axons have been identified, filled, and traced to the three otoconial maculae or the two auditory papillae (Table 2).

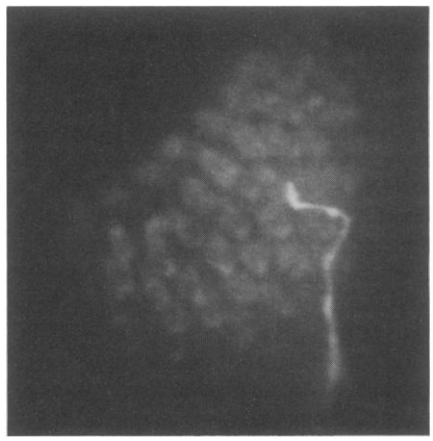

Fig. 1. Photomicrograph of a fluorescing auditory afferent axon projecting to a single hair cell in the third row from the medial edge of the basilar papilla. The autofluorescing hair cells (with dark nuclei) provide excellent landmarks for locating individual afferent terminations in a sensory surface.

Our studies have confirmed that the bullfrog's higher frequency auditory sensitivity (best excitatory frequency in the range 1000 to 2000 Hz) resides in the basilar papilla and that its lower frequency auditory sensitivity (best excitatory frequency in the range 100 to 1000 Hz) resides in the amphibian papilla and the saccule. We found that within the amphibian papilla frequency sensitivity is distributed tonotopically, with the lower frequencies (100 to 300 Hz) at the rostral

Table 2. Peripheral origins of sensitivities in the bullfrog inner ear as determined by dye-tracing studies.

Sensitivity	Peripheral origin	Hair cells innervated		Number of axons identified and traced
		Number	Type (*2*)	
Auditory				
> 1000 Hz	Basilar papilla	1 (rarely 2 to 4)	D, F	22
600 to 1000 Hz	Amphibian papilla (caudal sixth)	1 to 6	D, E	6
300 to 600 Hz	Amphibian papilla (middle half)	1 to 8	A, D, E	12
< 300 Hz	Amphibian papilla (rostral third)	1 to 15	A, D, E	13
Auditory-seismic				
< 300 Hz	Entire saccular macula	30 to 200	D	5
Seismic	Entire saccular macula	2 to 30	A, D	6
	Lagenar macula (center of central field)	6 to 10	E	13
Seismic-vestibular (*16*)	Lagenar macula (center and edge of central field)	6 to 10	C, E	5
Phasic vestibular (*16*)	Utricular and lagenar maculae (between edge and center of central field)	10 to 15	F, some C	9
Phasic-tonic vestibular	Utricular and lagenar maculae (edge of central field)	10 to 15	C, some F	11
Tonic vestibular	Utricular and lagenar maculae (peripheral field)	6 to 20	B	7

end, the higher frequencies (600 to 1000 Hz) at the caudal end, and the intermediate frequencies in the intervening region. We found that seismic sensitivity is present over the entire saccular macula and over a narrow band in the center of the lagenar macula. Sensitivity to linear acceleration (for example, tonic gravitational sensitivity) was found over the large peripheral fields of the lagenar and utricular maculae. Sensitivity to changes in linear acceleration (for example, phasic gravitational sensitivity) was found within the narrow central fields of those maculae, directly beneath the striolae of the otoconial membranes.

Lewis and Li (*2*) lumped the hair cells of the bullfrog into six types based on surface topography and presented maps of the distributions of those types over the three maculae and two papillae. With those maps it was relatively easy to determine which hair-cell types were associated with each terminal arborization (Table 2). If we assume that the five seismic-vestibular lagenar units identified and traced so far owe their seismic sensitivity to the type E hair cells that they innervate and their vestibular sensitivity to the type C hair cells, the following correspondences between hair-cell type and function emerge from Table 2. (i) Seismic and auditory sensitivities are associated with hair-cell types A, D, and E. (ii) Phasic vestibular sensitivity is associated predominantly with hair-cell type F. (iii) Phasic-tonic vestibular sensitivity is associated predominantly with type C. (iv) Purely tonic vestibular sensitivity is associated with type B. Only the seismic or auditory hair cells (types D and E) possess kinociliary bulbs.

No tonotopic organization has been recognized so far in the basilar papilla. The clear tonotopic organization in the amphibian papilla is interesting inasmuch as that organ lacks a basilar membrane, the structure presumed to be at least partially responsible for tonotopy in the mammalian cochlea. The bullfrog is the third lower vertebrate shown to have auditory tonotopy; others are the alligator lizard and the granite spiny lizard (*17*). In the bullfrog amphibian papilla, the low-frequency region is directly adjacent to the thickest (and therefore presumably most massive) portion of the tectorium, while the high-frequency region is directly adjacent to the thinnest (presumably least massive) portion. These are the associations one would expect if the tectorium were serving as part of a distributed mechanical filter producing the tonotopy.

The seismic (vibratory) sensitivity of the saccular macula is especially acute (for example, in one saccular axon, responses could be seen to sinusoidal stimuli of $2 \times 10^{-5}g$ peak acceleration, or approximately 1 nm of substrate displacement, in the neighborhood of 100 Hz). This acuteness may account for the sensitivity observed in some saccular axons not only to seismic vibration but also to airborne sound, which inevitably is coupled to the substrate and thus produces some seismic vibration. To date, no axons have been traced to the center of the central field in the utricular macula. Therefore, the presence or absence of seismic sensitivity in that organ remains in doubt. We have also been unable to identify sensitivity variations corresponding to the mediolateral morphological variations in the auditory papillae or sensitivity differences between the central and peripheral fields in the sacculus.

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Spinal Sympathetic Neurons: Possible Sites of Opiate-Withdrawal Suppression by Clonidine

Abstract. *Morphine, methadone, meperidine, fentanyl, and clonidine rapidly depressed transmission through sympathetic preganglionic neurons in cats with the spinal cord transected. Naloxone promptly antagonized this effect of the opiates but not that of clonidine which was reversed by α_2-adrenergic receptor antagonists. The independent depression of preganglionic neurons by clonidine may contribute to the ability of this drug to depress the symptoms of opiate withdrawal that are characterized by sympathetic hyperactivity.*

Clonidine, a potent centrally acting antihypertensive drug (*1*), is remarkably effective in reducing the symptoms of opiate withdrawal in man (*2*) and animals (*3, 4*). Although clonidine does not share the addiction liability of the opiates, both drugs produce sedation, analgesia, respiratory depression, bradycardia, and hypotension (*1, 4–7*). Furthermore, abrupt discontinuation of long-term therapy with clonidine can produce symptoms that closely resemble (*8*) but are milder than those precipitated by withdrawal from opiates (*2, 5*). Many features of these withdrawal syndromes indicate hyperactivity of the sympathetic nervous system, possibly because of a rebound hyperexcitability of some central neu-

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