evidence for both intraspinal and supraspinal sources. We observed enkephalin cells after colchicine treatment throughout the dorsal horn, especially in the substantia gelatinosa and at the junction of laminae II and III. A small number of enkephalin-labeled cells were found in the sympathetic nuclei intercalatus spinalis and pars paraependymalis. Previous studies (*7, 8, 10, 11, 21*) showed enkephalin-labeled cells in the dorsal horn, intermediate zone, and dorsal to the central canal, although the majority of immunoreactive cells were found in the substantia gelatinosa (*7, 8, 10*). Transection of the spinal cord at thoracic levels or unilateral dorsal rhizotomy at lumbar or sacral levels does not produce changes in the distribution of enkephalin within the spinal cord (*8*), and this suggests an intraspinal origin for enkephalin fibers. However, the results of a study (*27*) in which a combined retrograde transport–immunocytochemical technique was used in the rat showed enkephalin projections from the medulla to the lower thoracic–upper lumbar regions. In corroboration, no enkephalin-immunoreactive staining was found in the thoracic cord after a C_7 spinal cord transection (*25*). Therefore, both intraspinal and descending enkephalin projections may exist, although the relative contribution of each to laminae VII and X is unknown.

Our results demonstrating that enkephalin fibers and sympathetic preganglionic nuclear regions are in the same place, provide an anatomical substrate at a spinal level for a functional relation between the enkephalin peptide and the sympathetic nervous system. More specifically, our findings may provide a morphological basis for the hypothesis that the augmented sympathetic activity during opiate withdrawal occurs at a spinal level. In a more general sense, our observations indicate that the integration of autonomic reflexes at a spinal level is mediated, at least in part, by an enkephalin system.

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Sex Ratio of Sea Turtles: Seasonal Changes

Abstract. *Sex ratios of hatchling loggerhead turtles* Caretta caretta *taken from South Carolina and Georgia ranged from no females in nests laid in late May to 80 percent females in those laid in early July; the sex ratio decreased to 10 percent females in nests laid in early August. These seasonal changes are consistent with the role of temperature in directing sexual differentiation in various reptiles. The data have implications for understanding the demography of sea turtles and for their conservation.*

Sexual differentiation in sea turtles, as in a number of reptiles, depends on the ambient temperature during incubation of the eggs (*1–3*). Therefore the sex ratio of offspring should differ at different times of year. This is especially likely to happen in species of sea turtles that lay several clutches over an extended nesting season. This idea has been discussed (*2, 3*), but conclusive data are lacking. We now report that seasonal changes occur in the sex ratio of loggerhead turtles (*Caretta caretta*) nesting in the southeastern United States. The effects are large and have implications for conservation programs and for the study of sea turtle demography.

Hatchling loggerhead turtles were collected from 1979 to 1982 from various barrier islands in South Carolina and Georgia. The nesting beaches frequented by loggerhead turtles in these regions are predominantly primary dune, either devoid of cover or sparsely covered with sea oats (*Uniola paniculata*). The lack of dense vegetation and the associated shade, along with the relative openness and homogeneity of the barrier island beaches, reduces the importance of spatial variables. From each clutch sampled, ten hatchlings were taken at random (*4*). Sex was determined histologically (*5*).

Sex ratio ranged from 10 percent female or less during the cooler ends of the season to 80 percent female in the middle of the summer (Fig. 1). Although variability occurred among clutches, none was less than 40 percent female between 12 June and 14 July, and most were 75 percent female or more. Not all the data came from the same year. When the results for 1982, the year with most available data, are considered separately, the seasonal trends are essentially the same. Also, some nests had been transplanted to protected sites soon after laying. However, there is no evidence that these nests had markedly different sex ratios (Fig. 1). This is not surprising because the areas selected for reburying the eggs were similar to those selected by nesting turtles.

Because seasonal frequency of nesting is known for these beaches, the overall sex ratio for the whole season may be estimated by combining the sex ratio and nesting frequency data (Fig. 1, A and C). When all the sex ratio data are combined with the average nesting frequency data for 6 years (1977 to 1982) for Sand and South Islands, 56.3 percent of the hatchlings are female (*6*). When similar calculations are made from the 1982 data only, 48.2 percent of the hatchlings are female. Because relative nesting frequencies over the season were similar among the different beaches and different years, estimates of the overall sex ratio are not greatly influenced by the particular data used for nesting frequency.

Sex ratios close to equality in species whose sexual differentiation is unconstrained by sex chromosomes (*7*) appear to provide a new form of confirmation of Fisher's (*8*) theory of equal parental investment in the two sexes (*9*). However, some cautions are in order. First, the estimate of overall sex ratio is only an approximation because there is some uncertainty about the sex ratios during different parts of the season (Fig. 1B). Second, it is conceivable that our sample did not adequately reflect relative nesting frequencies on differently oriented faces of dunes with subtly different thermal characteristics. Third, there are indications that on some other beaches sex ratios may be unequal (*10, 11*), although these data cannot be properly assessed without information on nesting frequency and sex ratio over the full season. Moreover, unequal sex ratios might be found for a particular beach because it may represent only part of a larger and thermally more diverse breeding area. If the tendency of adults to return to a particular site for nesting is weak, then the population as a whole may not be represented fairly in samples from one beach (*11*). More extensive work with turtle populations nesting in thermally different regions is needed to determine whether the present estimates of equality of investment in the two sexes are meaningful or just chance. Of course, the sex ratios of juveniles or adults might differ from those given for hatchlings; differential mortality during different parts of the season could result in skewed sex ratios.

We present conclusive evidence of seasonal trends that will have to be taken into account in any tests of Fisher's (*8*) theory. Similar investigations could also be made in areas where turtles nest year round. It has been suggested that two different turtle populations nest at some of these locations (*12*). Study of year-round changes in sex ratio could be used to validate these speculations (*2*).

Our findings also have implications for turtle management and conservation. For instance, it has been proposed that, if eggs are harvested, they should be taken early in the season because of the greater chance that some of the early nests will be destroyed by turtles nesting later (*13*). Such selective harvest of early nests could distort sex ratios. Also, the practice of starting nest protection projects only when turtles are nesting in reasonable numbers, although economic in terms of manpower, might affect sex ratios in the long run. Finally, these data should be taken into account in attempts to solve problems in turtle demography with the use of "living tags" [transplanting slivers of pale plastron tissue to the darker carapace (*14*)]. More tag returns are likely if females are tagged. That means tagging hatchlings from eggs laid in the middle of the season.

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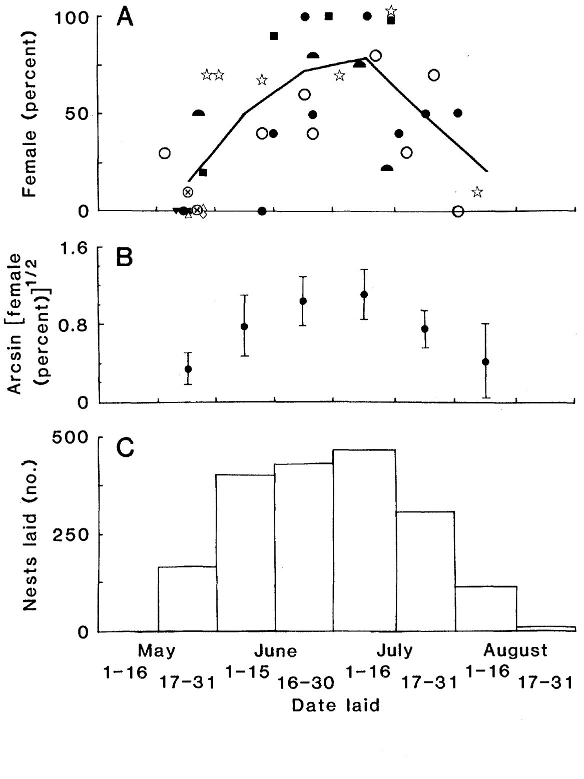

Fig. 1. (A) Percent female of samples (ten hatchlings) from clutches laid on beaches in South Carolina and Georgia. The line shows the mean for clutches laid in each half-month of the nesting season. Solid symbols are for clutches left in situ: (▼) Sand Island, South Carolina, 1979; (■) South Island, South Carolina, 1980; (●) South Island, South Carolina, 1982; (▲) Kiawah Island, South Carolina, 1982. Symbols with open centers are for reburied clutches: (◇) Fripp Island, South Carolina, 1979; (⊗) Ossabaw Island, South Carolina, 1979; (△) Little Cumberland Island, Georgia, 1979; (☆) Little Cumberland Island, Georgia, 1980; (○) South Island, South Carolina, 1982. (B) Means ± 2 standard errors (in radians) for clutches laid in each half-month of the nesting season. A standard arcsin transformation was used because percentages were not normally distributed. (C) Nesting frequency for Sand and South Islands combined for 1977 through 1983.

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true; it might cost more for turtles to lay at certain times of the year or to climb higher on the beach into a shady area. However, with similar egg sizes it is reasonable to assume that the costs of producing a male or female are close.

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Ontogenetic Changes in Frequency Mapping of a Mammalian Ear

Abstract. *Cochlear microphonic iso-response functions reported here suggest an explanation of frequency-dependent changes in hearing sensitivity during early development. The work is a direct demonstration of developmental changes in the spatial frequency map of the mammalian hearing organ. Intracochlear recordings from the midbasal turn in a series of age-graded gerbils reveal a progressive increase in best frequency, spanning approximately two octaves, from the time of onset of function until adultlike responses are seen. It is, therefore, suggested that ontogenetic changes in the cellular structure of the organ of Corti contribute to an age-dependent shift in micromechanical response.*

Puzzling discrepancies are evident during development between sequential anatomical changes in the cochlea on the one hand and concomitant physiological measures on the other. The structural maturation of the cochlea progresses from the basal (high-frequency) end of the structure toward its apical (low-frequency) end. For example, differentiation and innervation (*1–5*) of the mammalian cochlear receptor cells first occur in the basal turn and progress apically. The same sequence has been reported for the morphological development of the supporting structure of the organ of Corti, including the opening of the tunnel of Corti, spaces of Nuel, and the inner spiral sulcus (*1, 3, 6–9*). In contrast, behavioral and physiological measures of auditory abilities at the time of the onset of function indicate that responses are first elicited by stimuli in the low- to middle-frequency range for any particular species studied (*9–11*). Since it has been well established that in the adult the basal segments of the cochlea preferentially respond to high-frequency stimuli while more apical portions are tuned to lower frequencies (*12, 13*), there seems to be a contradiction between the anatomical and the functional data. It has been suggested that selective attenuation of high frequencies by the immature middle ear may explain this conflict (*14*). Several investigators proposed that developmental changes in cochlear, in addition to middle-ear, mechanics underlie the discrepancy (*3, 11, 15*). Most notably, Rubel and his colleagues (*9, 16*) have suggested, on the basis of work with avian ears, that the immature basal portion of the cochlea is maximally responsive to low or low-middle frequencies. It is assumed that the place of maximum sensitivity to a specific frequency shifts apically during development while the basal portion develops sensitivity to progressively higher frequency stimuli. We report an experiment performed on mammalian ears that supports this theory.

If the pattern of frequency sensitivity along the cochlear partition changes during development, as Rubel suggests, then the best frequency at a specific location should increase systematically from the time of functional onset of hearing until adultlike responses are observed. To test the validity of these notions in the mammalian ear, it is essential to eliminate the influence of the maturing middle ear on the measurements. We used the following strategy that is insensitive to the state of middle ear transmission. The best frequency at a given cochlear location was determined from the frequency response pattern of the cochlear microphonic (CM) potential. To this end, intracochlear electrodes were placed in the midbasal turn in a series of age-graded Mongolian gerbils (*Meriones unguiculatus*) (*17*). The tuning properties were determined from CM iso-response functions (*13*) and referenced to similar functions recorded from the round window. This maneuver (*18, 19*) provides a measure of tuning that depends primarily on the frequency re-

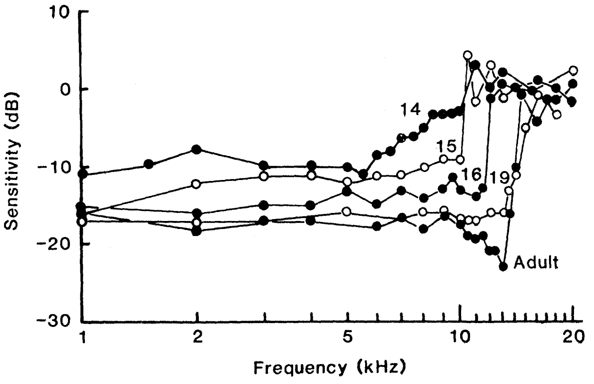

Fig. 1. Examples of sensitivity patterns obtained in individual gerbils as a function of stimulus frequency. The age of the animal in days is indicated on each curve. These functions are obtained as follows. An intracochlear electrode (24 μm diameter tungsten wire insulated with glass) was placed in the scala tympani of the first (basal) cochlear turn. Another electrode, silver wire, was placed on the round window membrane. The indifferent electrode was a common location on the metal head-holding device. Electrical signals were amplified in separate wide-band amplifiers, displayed on an oscilloscope, and measured with a 3-Hz bandwidth wave analyzer. Sound was delivered to the animal's ear by a headphone assembly (Beyer), an integral part of which was a probe-tube microphone for measuring sound pressures in the immediate vicinity of the eardrum. The data were obtained in terms of the sound pressure level necessary to elicit a criterion (1 μV) CM response at any given frequency. Sensitivity was computed as the decibel difference between the first turn and the round window readings. Thus a negative number indicates greater sensitivity (lower sound pressure level) at the intracochlear recording site. Note that all functions, including that depicting adult behavior, show a high-frequency plateau. The level of this flat portion is largely determined by the noise level of the recording apparatus. Data points within this plateau for any animal were averaged, and the resulting value was arbitrarily designated as 0 dB. Thus the plots depict data normalized to the level of this high-frequency plateau. Before data collection, the animals were anesthetized (5 to 20 mg of ketamine per kilogram of body weight, dose increasing with age) and the auditory bulla was opened from a basolateral approach (*19*). In adults a tracheal cannula was placed, but in pups the natural airway was maintained. During surgery and experiments the animals were kept warm with the aid of a water blanket. In adults the hole admitting the intracochlear electrode was drilled by hand through the bony capsule (*13*). In young animals (up to 14 days) the capsule is cartilaginous and drilling is not possible. In these cases the hole was made by gently piercing the capsule with a fine needle.