reaction, which has always been regarded as the function of the flexor reflex. With this single reservation, the responses of the motoneurons that innervate the EUS may be called flexor reflexes.

This is apparently the first time it has been suggested that reflex impulses to nonlimb or nontrunk muscles can be classified as flexor or extensor on the basis of their pattern and distribution (*3*). Characterizing the responses of EUS motoneurons to cutaneous stimuli as flexor reflexes would, of course, not imply that all discharges of these motoneurons are flexor reflexes. For example, afferent impulses from the bladder, urethra, perineal skin, or genital mucosa may elicit responses in the nerves to the EUS that are not flexor reflexes (*10*).

Having established that, in terms of cutaneous stimuli, the motoneurons innervating the EUS respond like those supplying limb flexor muscles, we must still ask what causes the overactivity of the EUS in paraplegia in the absence of overt stimulation of the skin and subcutaneous tissues of the lower limbs. The motoneurons of the EUS apparently differ in their properties (*11*) from those supplying limb flexor muscles, and might be unusually susceptible to discharge. To determine whether impulses originating in skin and subcutaneous tissues in the absence of applied stimulation are sufficient to cause the maintained firing in the nerves to the EUS generally observed in paraplegia, we anesthetized the hind limbs. This abolished mechanically evoked flexor reflexes in the nerves to the EUS and greatly reduced the tonic efferent activity in them. It appears that these impulses are an important source of the excitatory drive to the motoneurons of the EUS in paraplegia or are essential in producing the discharge by which these cells cause EUS overactivity.

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2. Under deep ether anesthesia the common carotid and vertebral arteries were occluded bilaterally. After transection of the spinal cord, anesthesia was discontinued and respiration was maintained with a pump.
3. Stimulation of skin of the lower extremities in paraplegic patients may cause contraction of the anal and urethral sphincters [D. Denny-Brown and R. G. Robertson, *Brain* **56**, 397 (1933); R. A. Kuhn, *ibid.* **73**, 1 (1950)]. This effect may be so strong that an ongoing micturition can be stopped by evoking the plantar flexor reflex. Nevertheless, these findings as well as elegant clinical studies on urethrovesical function during spinal shock (*9*) and animal studies demonstrating contraction of the EUS during stimulation of hind limb skin [J. W. Downie and S. A. Awad, *Invest. Urol.* **17**, 55 (1979)] did not lead to recognition of the flexor reflex control of the EUS. J. Pedersen, H. Harving, B. Klemer, and J. Torring [*J. Neurol. Neurosurg. Psychiatry* **41**, 813 (1978)] noted, however, that both perianally and peripherally elicited reflexes of the anal sphincter have many features in common with the flexor reflex.
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10. Local anesthesia of perineal skin in paraplegic patients reduces the activity of the EUS (M. A. Sabbahi and C. J. De Luca, personal communication). Topical anesthesia of the urethral mucosa and submucosa reduces the current in anc tone of the EUS [E. Bors, A. Rossier, F Sullivan, *Urol. Surv.* **12**, 205 (1962)]. These experiments suggest that nonflexor inputs contribute to the tone of the EUS but are not sufficient by themselves to account for it.
11. The motoneurons that supply the EUS are spared in several diseases that destroy other motoneurons. T. Mannen, M. Iwata, J. Toyokura, and K. Nagashima [*J. Neurol. Neurosurg. Psychiatry* **40**, 464 (1977)] reported remarkable preservation of this group of motoneurons in amyotrophic lateral sclerosis, in which urinary function is almost always preserved throughout the illness. M. Iwata and A. Hirano [*Ann Neurol.* **4**, 245 (1978)] reported sparing of these motoneurons in Werdnig-Hoffmann disease and poliomyelitis. E. Pons Tortolla, R. Roca-de-Vinals, and B. Rodrequez-Arias [*Rev. Neurol* **85**, 165 (1951)] also reported sparing of these motoneurons in poliomyelitis. Whether these differences in susceptibility to disease are related to the persistent activity of these cells in paraplegia is not known.
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Temperature-Dependent Sex Determination: Current Practices Threaten Conservation of Sea Turtles

Abstract. *Temperature determines the sex of hatchling green turtles (*Chelonia mydas*) produced from eggs incubated in a beach hatchery under different temperature regimes. Cold and cool nests (< 28°C) produced almost no females (0 to 1(percent) and warm, thermostable nests (> 29.5°C) produced almost all females (9: to 100 percent). A few intersex hatchlings were produced at lower temperatures Since little concern is given to temperatures at which sea turtle eggs are incubated in artificial hatcheries, present conservation practices may be producing all male, al female, or even intersex hatchlings.*

Green turtles (*Chelonia mydas*) like many other sea turtles are in danger of extinction. Extensive conservation efforts in many countries include efforts to protect eggs by incubating them in central hatcheries on beaches and in artificial nests in Styrofoam boxes aboveground. Little concern has been given to temperatures at which the eggs are incubated (*1*). Female sea turtles come ashore, deposit their eggs into nests excavated in the sand, cover them, and return to sea. There is no parental care of the nest, and hatchlings emerge from the nest unassisted (*2*).

Turtles, in general, do not have heteromorphic sex chromosomes, and sex determination is dependent on the temperature at which eggs are incubated (*3–6*). Among freshwater turtles of several genera, cool temperatures (24° to 27°C) produce male offspring, and warm temperatures (31°C and above) produce female offspring (*5, 6*). This occurs both in the laboratory and under natural conditions (*7*), and this phenomenon has been the subject of recent reviews (*7, 8*). One study (*9*) suggests that sex determination in loggerhead sea turtles (*Caretta caretta*) is also temperature-dependent. If this is true for other species, then current conservation practices may be jeopardizing survival of sea turtles by producing all male or all female hatchlings.

Since green turtles lack heteromorphic sex chromosomes (*10*), we studied the

Table 1. Temperatures for 19 nests of green turtles in a beach hatchery at Tortuguero, Costa Rica. Measurements were taken daily throughout development; S.D., standard deviation.

Condition	Number of nests	Number of measurements	Temperature (°C)		
			Means ± S.D.	Minimum	Maximum*
Cold	5	247	27.9 ± 0.95	25.4	31.0
Cool	5	241	28.6 ± 1.21	26.3	32.1
Warm	4	225	31.4 ± 1.68	28.2	35.9
Warm and variable	5	228	31.4 ± 2.05	27.7	36.5

*Maximum temperatures were the result of metabolic heating late in development after sexes were determined.

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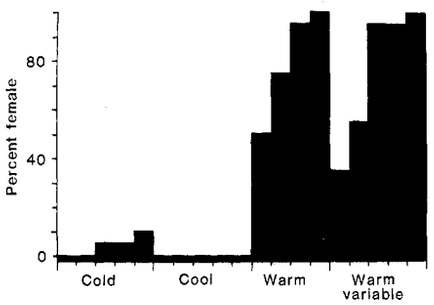

Fig. 1. Production of female hatchlings from nests in a beach hatchery at Tortuguero, Costa Rica, in four temperature regimes. Cold and cool nests produced predominantly males, and nests in warm areas produced females. Differential mortality did not affect the results because hatching success was high and similar in all four thermal regimes (cold, 80 to 100 percent; cool, 77 to 98 percent; warm, 89 to 99 percent; warm and variable, 87 to 98 percent). A few intersex individuals were found (6 of 100 in cold nests and 2 of 80 in warm nests). These may have been the result of incomplete sex determination caused by incubation of natural nests at low or intermediate temperatures. Complete clutches of eggs (mean = 104, range 50 to 147) were obtained from nesting females by collecting eggs in clean plastic bags as they were being deposited. We made artificial nests of the same dimensions as natural nests and buried eggs within 6 hours after collection (five nests for each thermal regime). Nest (center) and control temperatures were monitored daily (± 0.1°C) with 24-gauge thermocouples and a portable thermocouple meter (Bailey, BAT 12). At hatching, a random sample of 20 hatchlings was chosen from each nest and killed by injection of 0.1 ml of euthanasia solution (T 61, National Laboratory Corp.) by cardiac puncture. Death was instantaneous. All other hatchlings were held until night and released into the sea. Hatchlings escaped from one of the warm nests before they were sampled. We dissected out kidneys and attached gonads from sample hatchlings and preserved these organs in 10 percent buffered Formalin. Histological sections were prepared of gonads and stained with hematoxylin and eosin. Slides were randomized, and sex determinations were made by a blind protocol, following criteria of Bull and Vogt (*6*) and Yntema and Mrosovsky (*9*). Details of our procedures are given elsewhere (*13*).

effect of temperature on sex determination in eggs of this species. During the summer of 1980 we constructed a hatchery on a natural nesting beach at Tortuguero, Costa Rica. Within a fenced enclosure (8 by 4.5 m) we established four thermal zones: the cold zone was totally shaded by palm thatching and nests were buried at normal depth (50 cm to the top of the nest); the cool zone was 50 percent shaded by palm thatching and nests were at normal depth; the warm zone was exposed to full sunlight and nests were at normal depth; and the warm and variable zone was exposed to full sunlight and nests were at half the normal depth (25 cm to the top of the nest). The first two regimes simulated conditions for natural nests located in vegetation at the edge of the beach, the third simulated conditions on the open beach where there was no vegetation, and the fourth was included to provide warm and more variable temperatures (Table 1).

Temperature did affect the sex of hatchlings produced from eggs incubated in the hatchery under the different temperature regimes. There was a statistically significant difference in the numbers of females produced from nests in the four thermal regimes [one-way analysis of variance, $P \leq .0001$, $F(3, 15) = 27.06$]. Cold and cool nests produced predominantly males (80 to 100 percent), and warm nests produced predominantly females (50 to 100 percent) (Fig. 1). Shallow nests (warm and variable) produced 35 to 100 percent females. Laboratory experiments suggest that the middle third of development is the critical time for sex determination in freshwater turtles (*5, 6*). Of nine nests where temperatures were above 29.5°C during this period of development (Fig. 2), five produced 95 to 100 percent females and four produced 35 to 75 percent females. The last four nests are discussed below.

Two of the warm, shallow nests produced 35 and 55 percent females. This may have been due to a cold shock at an early stage of development. The top of the egg chamber in shallow nests was only 25 cm from the sand surface. Eggs in these nests experienced more variation in temperature (Table 1) than those in nests at normal depth (50 cm to the top of the egg chamber). There were several days of rain and overcast skies in late August when eggs in the shallow nests were entering the middle third of development. Temperature in the center of one shallow nest dropped from 30.3°C to between 28.1° and 28.4°C, and temperature in the other dropped from 30.1°C to between 27.7° and 28.3°C for 3 days. Thermal profiles indicated that eggs near the top of the nests experienced even lower temperatures. This pulse of low temperatures may have induced maleness in many embryos. Male determination is more easily induced than female determination in freshwater turtles (*Graptemys*), and an isolated pulse of low temperature can have a substantial male-inducing effect (*7*). Eggs deeper in the nests and those in nests at normal depth were not as affected by this transient drop in surface temperature, remained warmer, and developed into females. During this same period three other shallow nests experienced only 1 or 2 days of temperatures near 28°C (27.9° to 28.2°C, 27.8° to 28.3°C, and 28.1° to 28.3°C). This pulse of cold was not intense enough or of sufficient duration during the critical stage to induce maleness since these three nests produced 95 to 100 percent females.

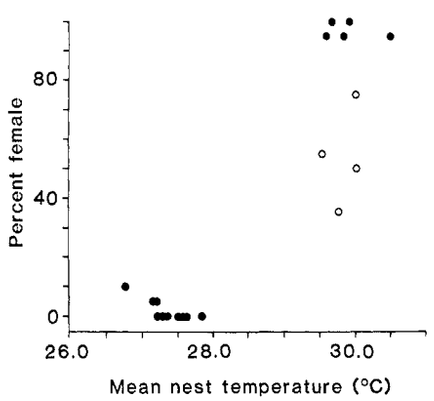

Fig. 2. Relation of nest temperature during the middle third of development to the percentage of female hatchlings produced in each of 19 nests. Open circles indicate nests that were subjected to cold shocks of varying intensity and duration during sex-determining stage of development.

One warm nest at normal depth produced 50 percent females and another, 75 percent females. Early in the middle third of development the first nest (50 percent females) had 4 days of temperatures of 28.7° to 29.2°C, and the second nest (75 percent females) had 4 days of 29.1° to 29.4°C. These nests were deep enough to be well insulated from daily fluctuations in surface temperature. Sand temperatures at these depths typically did not vary more than 0.5° to 1.0°C daily. Moderate reduction in nest temperature during the rainy period may have held the embryos at an intermediate temperature long enough to allow determination of maleness in some individuals. Metabolic heating began midway through development, shortly after the cool rainy period. Eggs in the center of these nests may have been warmer and developed into females while those on the periphery remained cooler and became males. This effect would depend on the shape of the egg chamber and thermal properties of surrounding sand. Data on this phenomenon are being analyzed.

Incubation of eggs in Styrofoam boxes produces males at low temperatures and females at high temperatures. Temperature also affects sex determination of eggs of the olive ridley turtle, *Lepidoche-*

lys olivacea (*11*). Thus, this phenomenon appears to be widespread and has direct implication for conservation efforts that involve artificial rearing of sea turtle eggs. Attempts to incubate eggs in hatcheries aboveground or in central beach hatcheries (*1*) should only take place after temperature-dependent sex determination is defined for the species in question. To disregard temperature is to risk producing all male, all female, or even intersex hatchlings. A beach hatchery is an effective way to incubate eggs in order to produce natural sex ratios if care is taken to duplicate as closely as possible the depth, the amount of shading, and egg chamber dimensions of natural nests. A thermal transect of the beach from water's edge into vegetation, taken at nest depth, will indicate where to locate the hatchery so that temperature ranges will produce desired sex ratios among hatchlings. Finally, in the absence of data on temperature-dependent sex determination and a thermal transect of a beach, artificial hatcheries should not be used. Rather, efforts should be directed to marking natural nests as they are made and to enclose them in wire mesh fences as soon as possible. Under these circumstances, protecting these natural nests from human and natural predators offers the best opportunity of enhancing production of large numbers of hatchlings while maintaining a natural sex ratio (*12*).

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Micromolar Affinity Benzodiazepine Receptors: Identification and Characterization in Central Nervous System

Abstract. *Receptors that selectively bind micromolar concentrations of benzodiazepines are present in rat brain membrane. These micromolar receptors exhibit saturable, stereospecific binding, and the potency of benzodiazepine binding to these receptors is correlated with the ability of the benzodiazepines to inhibit maximum electric shock–induced convulsions. Benzodiazepine receptors with nanomolar affinity differ from the micromolar receptors in their binding, kinetic, and pharmacologic characteristics. The micromolar receptors also bind phenytoin, a non-benzodiazepine anticonvulsant. These results provide evidence for a distinct class of clinically relevant benzodiazepine receptors that may regulate neuronal excitability and anticonvulsant activity.*

Benzodiazepines are widely administered therapeutic drugs with diverse clinical applications (*1*). Several of the pharmacologic effects of the benzodiazepines have been attributed to a class of well-characterized, stereospecific receptors that selectively bind benzodiazepines in the nanomolar range (*2–5*). Although these receptors appear to mediate some of the clinical effects of the benzodiazepines, several studies show that they do not account for the complete range of benzodiazepine therapeutic actions (*4, 5*). In particular, the potency of benzodiazepine binding to these receptors is not significantly correlated with the ability of the benzodiazepines to inhibit maximum electric shock–induced convulsions in animals or their ability to alter the behavior of animals in conditioned avoidance tests (*4, 5*). These observations suggest that other benzodiazepine receptors are present in brain.

The existence of a distinct class of benzodiazepine receptors with binding affinities in the micromolar range is suggested by studies demonstrating that micromolar concentrations of benzodiazepines in the brain are pharmacologically active (*6*). Furthermore, micromolar concentrations of benzodiazepines bound to brain membrane inhibit Ca^{2+}-calmodulin protein kinase activity (*7*). We have now identified and characterized a stereospecific benzodiazepine receptor that has micromolar binding affin-

Table 1. Differences in binding and pharmacologic characteristics for the micromolar and the nanomolar benzodiazepine receptors in brain membrane.

Characteristic	Nanomolar receptor	Micromolar receptor
K_D (M)	3.1×10^{-9}	4.5×10^{-5}
B_{max} (pmole per milligram of protein)	0.893	360.4
Stereospecific binding	Yes	Yes
Dissociation rate constant, k_{-1} at 4°C (sec^{-1}) (*14*)	2.69×10^{-3}	5.50×10^{-3}
Association rate constant, k_{+1} at 4°C (M^{-1} sec^{-1}) (*14*)	1.13×10^{6}	1.07×10^{2}
Pharmacologic correlation (*17*)		
Maximum electric shock–induced seizures	No	Yes
Muscle relaxant activity	Yes	No
Mouse rotorod performance	Yes	No
Pentylenetetrazol-induced seizures	Yes	No
Conditioned avoidance tests	No	No
Relative affinity for Ro5-4864 (*16*)	> 10,000	5.7
Enhanced binding (*15*)		
$10^{-4}M$ GABA	Yes	No
$10^{-4}M$ muscimol	Yes	No

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