Our results show that benzodiazepine receptor ligands may interact with the receptors in different ways, producing a spectrum of pharmacological effects. Some barbiturates may represent a similar continuum of receptor ligands interacting with their target in different ways (*19*).

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Flexor Reflex Control of the External Sphincter of the Urethra in Paraplegia

Abstract. *In paraplegics and quadriplegics a profound paralysis of skeletal muscles occurs below the level of the spinal lesion. Unexplained in this state is the development of an overactive external urethral sphincter, which interferes with emptying of the bladder and may lead to infection of the urinary tract. Studies of cats show that the discharge of motoneurons causing this contraction has all the characteristics of a flexor reflex.*

In paraplegia and quadriplegia of an upper motoneuron type, overactivity of the external urethral sphincter (EUS) develops, preventing the passage of urine. The discharge of the motoneurons responsible for this overactivity has been poorly understood. It has long been known that certain natural or experimental stimuli below the level of the spinal lesion affect the activity of the EUS. The reflex pattern of these effects and their functional significance in controlling the EUS, however, have never been adequately analyzed. In experiments on rats and cats, we noted that stimulation of cutaneous nerves in the hind limb elicits responses in ipsilateral nerves to the EUS similar to the discharges in flexor muscle nerves. Lloyd (*1*) classified the latter as flexor reflexes.

We report that these hitherto unclassified responses of the EUS and the efferent discharges in its nerves meet the accepted criteria for flexor reflexes in limb muscles. Local anesthesia of the skin and subcutaneous tissues of the hind limbs abolishes these flexor reflexes and the tonic efferent activity in the nerves to the EUS, suggesting that sensory inputs from these areas are essential elements in the overactivity of the EUS.

In experiments on cats whose spinal cords were transected at the obex (*2*), the same types of stimuli that elicited flexor reflexes in limb muscles evoked contractions in sphincter muscles. Nociceptive stimuli that evoked strong contractions in ipsilateral flexor muscles also elicited brisk contractions of the EUS. If one paw was pricked with a needle, for example, there was a sudden withdrawal of that limb due to contractions of flexor muscles in the hip, thigh, and leg. The same stimulus caused the EUS and anal sphincter to contract. Non-nociceptive stimuli—such as moderate heat, cold, pressure, and touch—applied to the skin of the thighs, legs, and feet evoked similar but less vigorous contractions in flexor muscles of the ipsilateral limb and also caused contractions of the urethral and anal sphincters. Electrical stimulation of these regions elicited almost simultaneous contractions of ipsilateral flexor muscles and both sphincters.

The distribution of reflex responses is one of the most important criteria in classifying them. Single-shock stimulation of cutaneous nerves (sural or superficial peroneal) in cats with high spinal lesions usually results in reflex contractions of flexor muscles in the ipsilateral hind limb. No contractions occur in ipsilateral extensor muscles, and responses evoked in these muscles by appropriately timed stimuli are generally inhibited during flexor reflexes. Contralaterally, the extensor muscles are excited and the flexors are inhibited.

Figure 1A shows three simultaneously recorded responses to stimulation of the left common peroneal nerve (a mixed cutaneous and muscle nerve). Trace a illustrates the absence of any reflex response in the ipsilateral gastrocnemius nerve (extensor); trace b illustrates a typical polysynaptic flexor discharge recorded from the ipsilateral biceps femoris nerve (flexor); and trace c is the response recorded from the nerve to the EUS. The reflex in the nerve to the EUS is part of a widespread response in a group of other pelvic floor and limb flexor muscles. These examples illustrate some of the similarities in the distribution of reflex responses in the nerves to flexor muscles and to the urethral and anal sphincters (*3*).

Another criterion in the classification of flexor reflexes is that they can be inhibited by (i) prior stimulation of nerves to direct antagonists or of some other extensor nerves and by (ii) elicitation of other types of ipsilateral extensor reflexes (*4*). Trace a in Fig. 1B illustrates the response of the nerve to the EUS to stimulation of the common peroneal nerve. Most of this large reflex was inhibited by delivering a single shock to the gastrocnemius nerve 10 msec before the test shock (trace b in Fig. 1B). The

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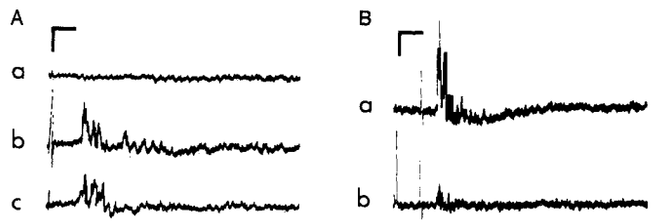

Fig. 1. (A) Simultaneous recordings from the proximal ends of severed nerves to the gastrocnemius muscle (a), biceps femoris muscle (b), and EUS (c), showing the reflex discharges elicited by a single shock to the ipsilateral common peroneal nerve. Calibrations: horizontal, 5 msec; vertical, 100 mV. (B) Recordings from the proximal end of the severed nerve to the EUS, showing reflex discharges elicited by a single shock to the ipsilateral common peroneal nerve (a) and inhibition of the same response (b) by a single shock to the ipsilateral gastrocnemius nerves delivered 10 msec before the test shock. Calibrations: horizontal, 5 msec; vertical, 100 mV.

response of the EUS was also strongly inhibited during an extensor thrust evoked by mechanical stimulation of the ipsilateral toe pads. When crossed extensor reflexes were elicited in the ipsilateral limb by any of a variety of electrical or mechanical stimuli applied to the contralateral limb, the response of the nerve to the EUS evoked by stimulation of ipsilateral skin or skin nerves was clearly inhibited. Thus, all the stimuli that inhibited the flexor reflexes of hind limb muscles also inhibited the reflexes of the EUS.

Classification of reflex responses in the nerve fibers supplying the EUS as flexor in type also requires that the electrical pattern of responses in these fibers be similar to that of flexor reflexes in limb muscle nerves. Returning to Fig. 1A, we see close similarity of the pattern in the flexor nerve (trace b) to the pattern in the nerve to the EUS (trace c). These two multipeaked discharges are in all respects similar to those Lloyd (*1*) classified as flexor reflexes.

Since all other spinal reflexes of cutaneous origin exert their effects on motoneurons only via internuncial cells (*5*), it was important to confirm the absence of monosynaptic connections between cutaneous receptors and EUS motoneurons. In a series of tests, no monosynaptic reflexes were recorded in the nerve to the EUS in response to stimulation of any nerve.

First, reflex discharges were elicited by brief shocks to the nerve to the EUS and recorded from distally severed ventral roots of the first, second, and third sacral nerves ipsilaterally. No monosynaptic reflexes were elicited by this stimulation, even when a 500-Hz conditioning tetanus was applied through the same electrodes prior to testing with single shocks. This absence of monosynaptic reflexes (*6*) confirms previous observations (*7*) and is consistent with reports (*8, 9*) that there are no muscle spindles or Ia fibers in the EUS. It was concluded that there are few if any monosynaptic connections from the EUS to its motoneurons. As controls for these observations, well-defined monosynaptic reflexes were elicited in the same ventral roots by stimulation of dorsal roots. Second, reflexes were recorded in the nerve to the EUS following stimulation of the seventh lumbar through third sacral dorsal roots. No monosynaptic reflexes were observed, but typical polysynaptic reflexes were produced by stimulating any of these dorsal roots. Finally, stimulation of the common peroneal, tibial, or sural nerves evoked only polysynaptic discharges in the nerve to the EUS. Thus, the pattern of electrical discharges recorded from the nerve to the EUS is in all respects similar to the pattern of reflex responses recorded from the nerves to limb flexor muscles.

We also studied the effects of passive stretching of flexor and extensor muscles on the EUS reflex. The animals were suspended in a frame by a clamp on both iliac crests, allowing easy manipulation of the hind limbs. The nerve to the EUS on the left side was placed on recording electrodes under mineral oil and the ipsilateral skin was stimulated electrically through wires in the subcutaneous tissue of the lateral side of the leg. This gave rise to a reflex discharge in the nerve to the EUS. Trace a in Fig. 2 illustrates the effect of placing both hind limbs halfway between full flexion and extension. With the ipsilateral limb fully extended (trace b), passively stretching most of its flexor muscles, the reflex was somewhat facilitated. Flexion of the contralateral leg had little effect, but contralateral extension (trace c) was clearly inhibitory. A return to the middle position is represented in trace d. Full flexion of the ipsilateral limb, passively stretching the extensor muscles, caused marked inhibition of the reflex (trace e). Combining the inhibitory effects from both limbs by flexing the ipsilateral leg and extending the contralateral one suppressed the reflex discharge of the EUS almost completely (trace f). The intermittent activity characteristic of the nerve to the EUS in paraplegic animals was also reduced with the limbs in this position. Similar results were obtained in rats transected at the midthoracic level by recording the activity of single anal sphincter motor units.

By every test, the discharges in the nerves to the EUS appear to qualify as flexor reflexes. Although the EUS is a midline muscle, the reflex discharges in the nerves innervating it on the two sides of the body follow the pattern of the flexor reflex in the strictest sense. Stimulation of cutaneous nerves elicits a reflex discharge only on the same side; contralateral stimulation inhibits it. Passive stretching of flexor muscles, moreover, facilitates the reflex discharge on the same side, whereas stretching of contralateral flexors inhibits it. The muscle fibers that form the EUS have no attachment to bone, and their contraction cannot produce even a localized withdrawal

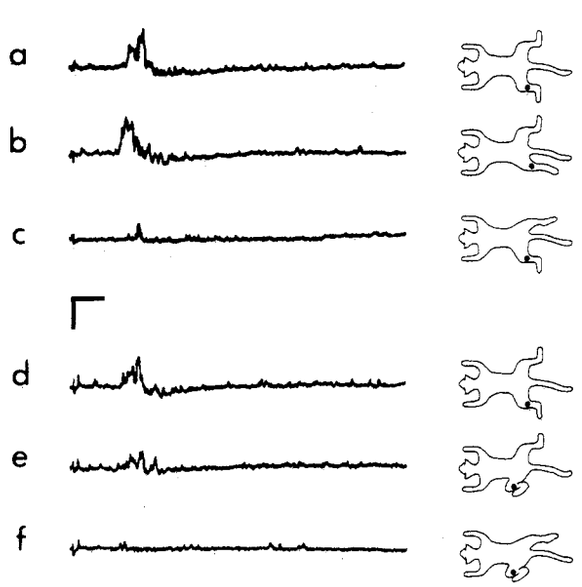

Fig. 2. Recordings of reflexes from the left nerve to the EUS, elicited by single-shock stimulation of subcutaneous tissue of the lateral side of the ipsilateral leg. The drawing accompanying each trace shows the position of the legs and the site of stimulation. Calibrations: horizontal, 5 msec; vertical, 50 mV.

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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