liter, exert no apparent effect on the behavior of the contracting cells. Larger amounts of calcium (above 100 μmole/liter) cause contracture. In addition to this unusual ionic milieu, the present system differs in several other potentially significant ways from the cardiac muscle cell cultures in which spontaneous contractions have been observed. Thus, the cells described here have not been exposed to trypsin, they initiate contraction immediately following disaggregation, and they are completely dependent on added (rather than endogenously produced) ATP. These qualities may prove of value for the study of heart muscle cell physiology and pathology.

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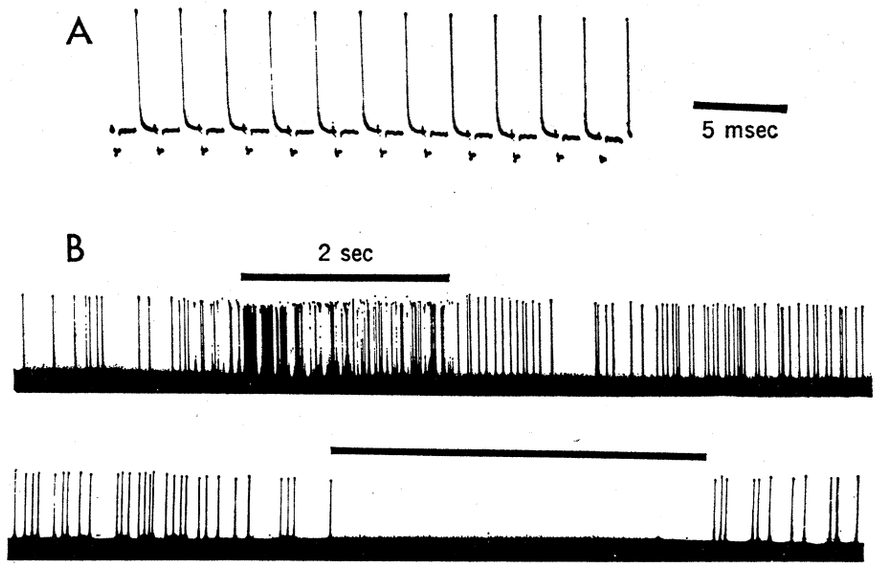

Fig. 1. Microelectrode recording from a direct somatovisceral fiber. (A) Electrical stimulation (20 volts, 0.5 msec) at C_1. Stimulus repetition rate, 250 per second. Note constant latency. (B) Typical excitatory and inhibitory responses to physiological stimuli. Bar above trace indicates duration of applied stimulus. (Top) Light brushing of hairs over contralateral thigh; (bottom) rapid injection of 2 ml of normal saline (37°C) into the gall bladder.

Somatovisceral Pathway: Rapidly Conducting Fibers in the Spinal Cord

Abstract. *Fibers that respond to distension of hollow viscera and to mechanical stimulation of somatic structures were found primarily near the ventromedian fissure of the upper lumbar spinal cord. These fibers could be directly excited by electrical stimulation at C_1. The average conduction velocity for these fibers was 68.6 meters per second.*

The locations of ascending exteroceptive and proprioceptive pathways in the mammalian spinal cord are well known. Both crossed and uncrossed tracts in dorsal, lateral, and the lateral part of the ventral columns have direct projections to higher centers. The locations of ascending visceral or interoceptive pathways, however, are not well known. With one exception (*1*), visceral pathways have been localized mainly in the dorsal and lateral columns.

Responses to visceral stimulation can be recorded from axons located throughout the ventral part of the spinal white matter (*2*). The present study was undertaken to determine whether these fibers project directly or indirectly to supraspinal loci. We now present neurophysiological evidence suggesting that in addition to propriospinal systems, there are rapidly conducting somatovisceral fibers which project directly to supraspinal loci. These fibers are located primarily in the ventral funiculus of the spinal cord along the median fissure.

The preparations (125 units obtained in 10 cats) were spinalized at the cervicomedullary junction and anemically decerebrated. Muscular paralysis and artificial ventilation were employed. Blood pressure, carbon dioxide concentration in expired air, and body temperature were monitored and maintained within physiological limits. Electrical stimuli were delivered through a transverse array of four electrodes placed in the cord just below the transection (C_1). Recordings were made at L_2 to L_4 with microelectrodes which contained 3*M* KCl and had impedances of about 20 megohm. The conduction distance from C_1 to the recording site was about 20 cm. Microelectrode tips were cut off in place and the tracts subsequently identified in frozen sections. Visceral stimulation

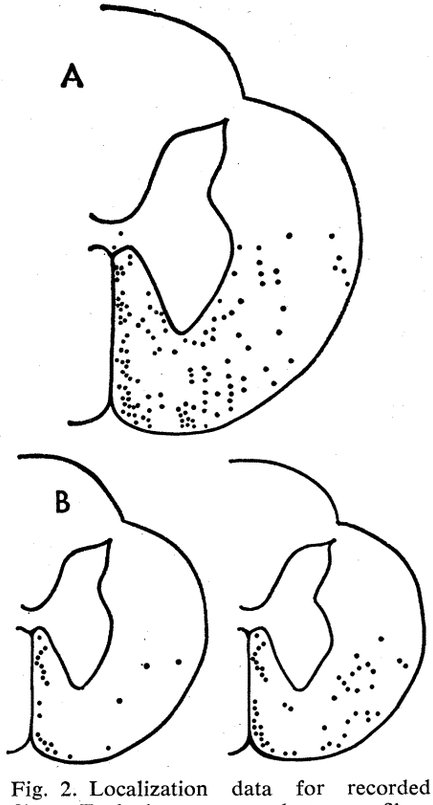

Fig. 2. Localization data for recorded fibers. Each dot corresponds to one fiber. The position was approximated by superimposing a photomicrograph of the section containing the microelectrode tip over a standardized L3 spinal cord cross section. (A) Total fiber sample. (B) (Left) Antidromically activated fibers with visceral inputs; (right) all antidromically activated fibers with detectable receptive fields or spontaneous activity, or both.

consisted of distension of the gallbladder and urinary bladder by injection of normal saline through implanted cannulae, and in some experiments, by balloon distension of the descending colon and rectum. Somatic stimulation consisted of mechanical manipulation of the extremities and body wall. Unit recordings were started between 6 and 30 hours after spinalization.

A unit was classified as a direct ascending fiber if a receptive field could be found and if it responded with a direct or antidromic spike in response to stimulation at C_1 (Fig. 1A). The criteria for establishing direct or antidromic activation included one spike for each stimulus (a following rate of at least 250 per second), invariant latency as stimulus frequency and intensity were varied, and, when present, collision between a spontaneously occurring spike and one evoked by C_1 stimulation. Units driven by stimulation at C_1, for which no receptive fields could be found and which exhibited no spontaneous activity were not classified since they could be either ascending, or more likely, descending fibers. Most units encountered, however, did have identifiable receptive fields but were not antidromically activated by cervical stimulation (*3*). These units are probably propriospinal.

Twenty-two units with visceral components in their receptive fields could be antidromically activated from C_1. These units were all spontaneously active and all responded to both somatic and visceral inputs (Fig. 1B).

Adequate receptive field studies were carried out in 18 of the direct somatovisceral fibers; 17 had bilateral and 13 had heterosegmental somatic inputs. The somatic receptive fields consisted of discrete, discontinuous components including cutaneous and deep structures. We found both excitatory and inhibitory fields, but we did not observe any systematic relation of these components. For example, the unit shown in Fig. 1B was inhibited by distension of both gallbladder and urinary bladder as well as by deep pressure over the middle third of the tail and lightly bending hairs in an area (1 by 2 cm) on the ventral metatarsal surface. This same unit was also excited by distension of the sigmoid colon, light touch over the sternum, light touch to the dorsal aspect of the ipsilateral thigh, and bending of hairs in an area (9 cm^2) on the contralateral thigh.

No units were found with purely inhibitory somatic inputs. Five had only excitatory somatic inputs; of these, two had inhibitory visceral inputs and three had excitatory visceral inputs. In six of the nine units that responded to both, the inputs from gallbladder and urinary bladder were of opposite sign. All six of these units had both excitatory and inhibitory somatic field components.

The average conduction velocity for 21 of the direct somatovisceral fibers was 68.6 ± 6.7 m/sec, with a range of 50 to 95 m/sec. These notably fast conduction velocities may not be representative inasmuch as our recording technique probably does not sample fibers conducting slower than about 30 m/sec (*4*, *5*). However, near the ventromedian fissure (Th 3), fiber diameters show a bimodal distribution (*6*). Whereas most fiber diameters are less than 7 μm, there is a secondary peak at 10.5 μm. This diameter peak corresponds to a velocity of 63 m/sec (*7*), a value consistent with our findings. A group of fibers that have a similar range of conduction velocities and demonstrate widespread convergence of somatic inputs has been found in the ventral part of the lateral funiculus (*5*); however, visceral inputs were not tested in these fibers.

The direct somatovisceral fibers tended to be near the ventromedian fissure (Fig. 2). The anatomical location, however, gives little clue as to the location of the pathway by which these fibers ascend the neuraxis. Although gross evoked potential and unit responses to visceral inputs have been found throughout the ventral white matter (*1*, *2*, *8*), most known ascending tracts are located in the dorsal or lateral funiculi. We have been unable to document an ascending pathway in the medial part of the ventral funiculus.

The above results demonstrate that there is a direct ascending pathway for visceral input. A highly convergent somatic input also projects to the fibers of this pathway. The presence of somatovisceral convergence at spinal neurons has been used as evidence to support the convergence projection theory of referred visceral sensation (*9*). Although the fibers we found do not ascend in classical somatosensory pathways, at least some fibers with both somatic and visceral inputs ascend to supraspinal structures.

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Persisting Circadian Rhythm of Cell Division in a Photosynthetic Mutant of Euglena

Abstract. *A persisting, free-running, circadian rhythm of cell division in a heterotrophically grown mutant of* Euglena gracilis *var.* bacillaris *having impaired photosynthesis is obtained upon placing a culture that has been previously synchronized by a 10,14 light-dark cycle into continuous darkness at 19°C (but not at 25°C). A similar persisting rhythm is initiated in exponentially increasing cultures (growing in darkness at 19°C) by a single "switch-up" in irradiance to continuous bright illumination. The results implicate an endogenous biological clock which "gates" the specific event of cell division in the cell developmental cycle.*

Entrainment of the cell division rhythm in *Euglena* by light-dark cycles or, perhaps, by temperature cycles and its persistence, after removal of the synchronizing regime, are thought to result from the functioning of an endogenous circadian clock that is intricately involved in the control of the cell cycle (*1*). We have assumed (*2*, *3*) that appropriate *Zeitgeber* entrain the cell division rhythm of individual cells in a population; under free-running condi-