labeled central cells within cervical and thoracic segments in numbers roughly equal to that labeled by LCN iontophoretic injections, they failed to label any such cells within the lumbar enlargement. Mesencephalic injections of HRP (two rats) labeled a few central cells ipsilaterally in the cervical enlargement, but none more caudally. These cells were found exclusively in the SDH. In the rat, but not in the monkey (*20*), thalamic HRP injections (six animals) failed to label any central cells.

Our findings, together with those of Willis *et al.* (*20*) and a similar observation in the trigeminal system (*21*), suggest that a significant number of the small cells of the SGR and SDH are involved in somatosensory processes in ways not suggested by earlier anatomic studies (*2-4*, *6*). However, a role for these cells may now be envisioned in modulating information transfer through sensory nuclei of the lower brainstem and upper cervical cord, comparable to that previously thought to occur only locally or at nearby spinal segments. These cells may also directly contribute information to sensory pathways of the brain in a manner more commonly associated with the larger projecting cells of the marginal zone and nucleus proprius. Finally, the far richer projection of central cells from the SDH than from the SGR gives further evidence (*1*, *5*) that although these areas are often considered as one on the basis of similar cellular morphology, they may prove to be functionally distinct.

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Spinothalamic Tract Neurons in the Substantia Gelatinosa

Abstract. *The substantia gelatinosa of the mammalian spinal cord is generally believed to be a closed system; that is, its neurons are thought to project only to the substantia gelatinosa of the same or the contralateral side. Experiments in monkeys, using injections of the marker enzyme horseradish peroxidase, show that at least some neurons of the substantia gelatinosa project to the thalamus and thus belong to the spinothalamic tract. Such neurons include two cell types intrinsic to the gelatinosa, the central cells and the limitrophe cells of Cajal.*

The substantia gelatinosa (SG) of Rolando was one of the first structures of the spinal cord to be recognized as a distinct entity (*1*). Although the exact circuitry formed within the SG by primary afferent fibers, axons descending from the brain, and the processes of neurons of the SG and the adjacent marginal zone and nucleus proprius is still under investigation (*2*), there is little doubt that the circuits are of importance for the central processing of data from nociceptors, thermoreceptors, and mechanoreceptors. For instance, speculation about the circuitry of the SG has led to at least one major hypothesis to account for inter-

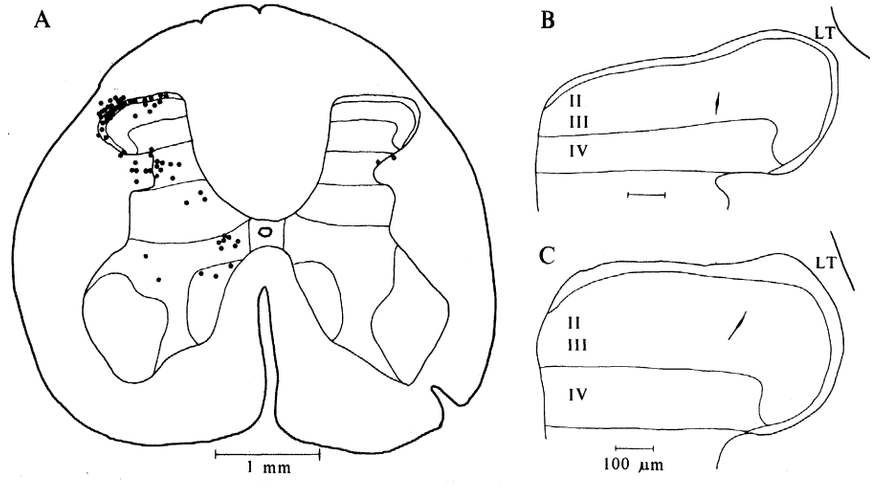

Fig. 1. (A) Distribution of spinothalamic tract cells in the lumbar enlargement of the spinal cord of a monkey. The cells were labeled by an injection of HRP into the region of the ventral posterior nucleus of the thalamus. Most of the cells were contralateral to the injection site. The cells shown were all those found in 15 consecutive alternate 50-μm sections in the L6 segment. Note that several cells were in the substantia gelatinosa. (B and C) Locations of two central cells in the substantia gelatinosa. Photomicrographs of the same cells are shown in Fig. 2, D and E. (The scale bars in B and C are 100 μm.)

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actions between the input to the spinal cord from mechanoreceptors and that from nociceptors. Although the details of the gate theory (*3*) of pain transmission have been challenged (*4*), it is clear that a detailed knowledge of dorsal horn circuitry is fundamental to an understanding of pain mechanisms.

The SG, according to Szentágothai (*5*), includes both laminae II and III of Rexed, although Rexed himself thought that the SG was coincident only with lamina II (*6*). The cell types to be found within the SG were described in detail by Cajal (*7*). These include the "cellules limitrophes" and the "cellules centrales." Limitrophe cells are located in the dorsalmost part of the SG and have dendrites that project ventrally in the SG. Central cells are fusiform cells distributed throughout the remainder of the SG. Their dendrites have an essentially radial orientation in transverse section and a rostrocaudal orientation in longitudinal section. These cell types have been confirmed by a number of investigators since Cajal (*5*, *8*). In addition, in the ventral part of lamina III, there are neurons with dorsally oriented dendrites, and there are various transitional forms (*8*). In addition to these intrinsic neurons, the SG also contains the dendrites of cells whose somata are located in the marginal zone or in the nucleus proprius (*7*). The primary afferent inputs to the SG include collaterals of fine axons that may first travel a short distance in the dorsolateral fasciculus of Lissauer, and the recurrent terminals of large myelinated fibers that penetrate the ventral aspect of lamina III (*7*). The small and large afferents appear to terminate on the intrinsic neurons of the SG and on the dendrites of the cells of the marginal zone and nucleus proprius. Presumably at least part of the information processed in the SG can be expressed through an output by tract cells located in the marginal zone and nucleus proprius. Deafferentation of the SG by dorsal rhizotomy, transection of the spinal cord above and below a segment 6 to 8 mm in length, and undercutting of the SG to eliminate input from deeper parts of the dorsal horn reveals that one projection target of the axons of the SG neurons is the ipsilateral SG (*5*). Another target demonstrated by Szentágothai (*5*), using the anterograde degeneration technique, is the contralateral SG. Cajal (*7*) felt that few of the cells of the SG have short axons that terminate close to the cell body. In recent studies (*9*), axons of SG neurons were traced in serial sections for distances of several millimeters before they could no longer be followed. This suggests that SG neurons are generally propriospinal neurons and leaves open the possibility that some SG neurons have long axons. Recently, we have obtained evidence that at least a fraction of the SG neuronal population projects to the brain. This observation requires a reassessment of current views of the circuitry of the SG.

For the work reported here, we used eight young macaque monkeys (*Macaca fascicularis*). The monkeys were anesthetized with sodium pentobarbital (30 mg/kg intravenously, with supplemental doses as required), and the marker enzyme horseradish peroxidase (HRP; Miles Laboratory) was injected into the thalamus on one side through a 26-gauge hypodermic needle by using a Hamilton microsyringe. The appropriate position for the injection was determined by recording the potentials evoked by natural stimulation of the skin or electrical stimulation of the sural nerve through the needle, which was insulated to its tip. The HRP was made up as a 50 percent solution, and the total volume injected ranged from 0.2 to 1.0 μl in one to four portions released at as many injection sites. After the HRP injection, the scalp was sutured closed, a prophylactic antibiotic injection (penicillin) was administered, and the animals were returned to their cages and allowed to recover from the anesthetic.

After 3 days, the animals were reanesthetized and perfused through the heart with 800 ml of Ringer solution followed by 800 ml of a mixture of 2.5 percent glutaraldehyde and 0.5 percent paraformaldehyde. The brain and spinal cord were removed, and fixation was continued in the same solution. After 8 to 24 hours, the tissue was blocked, rinsed, and placed in 30 percent sucrose. After 12 to 24 hours in sucrose solution, the tissue was sectioned on a freezing microtome at a thickness of 50 μm. The sections were reacted in H_2O_2 with either *o*-dianisidine or tetramethylbenzidine (*10*), mounted on slides, and stained with cresylecht violet or neutral red.

The injection sites in the thalamus

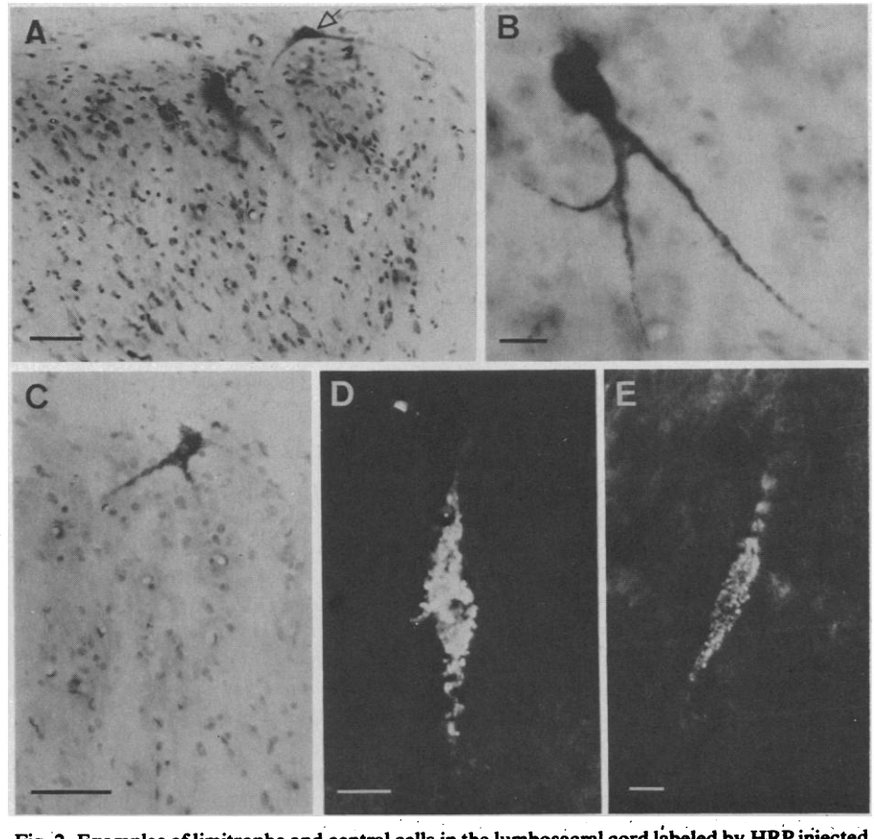

Fig. 2. Examples of limitrophe and central cells in the lumbosacral cord labeled by HRP injected into the contralateral thalamus. (A and B) Limitrophe cell at two different magnifications. (A) Lower-power view showing a marginal cell (open arrowhead) just dorsal to the limitrophe cell; the focal plane was adjusted to emphasize the substantia gelatinosa as demonstrated by a Nissl counterstain (scale bar, 50 μm). (B) Higher-power view at the focal plane for the limitrophe cell (scale bar, 20 μm). (C) Another limitrophe cell (scale bar, 50 μm). (D and E) Central cells in the locations indicated in Fig. 1, B and C (scale bars, 10 μm). The staining procedure for (A) to (C) was the *o*-dianisidine reaction, and the photomicrographs were made with bright-field illumination. The staining for (D) and (E) was with tetramethylbenzidine, and the photographs were made with dark-field illumination.

were reconstructed from serial alternate sections. The HRP reaction product was distributed in two zones: a large, weakly stained area and a smaller, heavily stained area. There is evidence that the area of active uptake of HRP by axon terminals coincides with the densely stained zone (*10*). However, it should be noted that even the larger weakly stained area in our animals never spread caudal to the mesencephalic-diencephalic junction zone.

To date, we have analyzed only the segments of the spinal cord from the fifth lumbar level caudally. A total of 4439 cells (average of 555 cells per animal) have been found to contain label that was transported retrogradely from the diencephalon. By definition, all such cells contribute to the spinothalamic tract. Many of the cells (23 percent) were in the marginal zone (Fig. 1A), a region that is rich in neurons responsive to input from nociceptors (*11*). Another area in which spinothalamic cells were concentrated was the region equivalent to Rexed's lamina V (*6*), and there were also spinothalamic cells in the intermediate region and ventral horn. These observations confirm the physiological evidence of such a distribution of spinothalamic cells in work done in our laboratory (*12*) and anatomic evidence from the HRP studies of several groups (*13*). An unusual finding in our study, however, was the size of the population of marginal cells that project to the diencephalon. Many of these cells were small and were often located in Lissauer's fasciculus or the adjacent portion of the marginal zone. We attribute our success in demonstrating such a large number of spinothalamic cells in the marginal zone to the use of the *o*-dianisidine and tetramethylbenzidine reactions, which appear to be more sensitive than the diaminobenzidine procedure (*10*).

A startling finding was the occasional observation of retrogradely labeled cells within the substantia gelatinosa (Fig. 1, B and C). A total of 50 such cells (1.1 percent of the population of spinothalamic tract cells observed) was found. However, this is an underestimate, since large cells in the region of junction between the SG and the nucleus proprius were excluded from this count. Most (47 SG cells) were contralateral to the thalamic injection site. Eight were relatively large and were not considered typical SG neurons. However, the remainder of the cells were typical of the neuronal population intrinsic to the SG. Although only the proximal dendrites could be visualized, the cells appeared to include both the limitrophe (25 cells) and central cell types (17 cells; see Fig. 2). Thirty-four of the cells were in lamina II and 16 in lamina III.

Although the fraction of the spinothalamic cell population that originates in the SG was small in these experiments, the presence of labeled spinothalamic cells in the SG was a fairly consistent observation in that we observed label in the SG of six of the eight monkeys studied. The negative findings in one animal can be accounted for by the fact that the HRP injection was placed too rostrally to label many spinothalamic cells of any type.

We conclude from our observations, those reported by Giesler *et al.* (*14*), and similar findings in the trigeminal system (*15*) that the mammalian substantia gelatinosa is not a completely closed system but instead includes cells that contribute to long ascending tracts.

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β-Endorphin Is Associated with Overeating in Genetically Obese Mice (*ob/ob*) and Rats (*fa/fa*)

Abstract. *Small doses of the opiate antagonist naloxone selectively abolished overeating in genetically obese mice* (ob/ob) *and rats* (fa/fa). *Elevated concentrations of the naturally occurring opiate β-endorphin were found in the pituitaries of both obese species and in the blood plasma of the obese rats. Brain levels of β-endorphin and Leu-enkephalin were unchanged. These data suggest that excess pituitary β-endorphin may play a role in the development of the overeating and obesity syndrome.*

The genetically obese mouse (*ob/ob*) and the Zucker fatty rat (*fa/fa*) display marked overeating, obesity, hyperinsulinemia, and transient or late-onset hyperglycemia. The syndrome is caused by a single recessive gene, *ob*, located on chromosome 6 in the mouse and a single recessive gene, *fa*, in the rat (*1*). The mechanism for the genetic expression of the syndrome is not known.

One striking difference between obese mice and their lean littermate controls is a 14-fold elevation of pituitary adrenocorticotrophic hormone (ACTH), a polypeptide, in the pituitary of 16-week-old animals (*2*). In addition, there is indirect evidence for elevated corticotrophin-like intermediate-lobe polypeptide (CLIP) (*3*). The ACTH and the CLIP share a common precursor molecule with another polypeptide hormone, β-endorphin. Thus, both β-endorphin and ACTH are

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