homologies are present because of selfing and, as a result, homoeologous pairing and segregation occur only occasionally. In the intraspecific hybrid, however, full homologies are not apparent and pairing behavior approaches randomness within a set of four homoeologous chromosomes. Greater similarity is apparent between some of the homoeologous chromosomes, however, because the segregation ratios indicate a low level of preferential pairing between chromosomes carrying the same allele. The pairing behavior described above appears to depend primarily on the degrees of homology rather than on genetic factors. For example, when full homologies were restored in the F_2 generation by selfing gametophytes from the F_1 hybrid, normal pairing behavior was restored uniformly within the F_2. In that cytological analyses have not shown the presence of multivalents in either the F_1 hybrid or within the inbred lines, the limitation of pairing to bivalent formation is evident. Whether this condition is a result of genetic or physical restrictions on pairing behavior remains unknown.

Intraspecific hybridization can be a significant force in promoting the release of stored variability within the described polyploid genetic system. Such hybridizations not only have the potential to generate new genotypic combinations but they also serve to release the variability stored within the duplicated loci through high levels of homoeologous pairing. Given this, and the maintenance of moderate levels of spore viability and full sporophyte vitality in the F_2 generation, the possibilities of producing new adaptive combinations are greatly increased over the situation within inbred lines. Because the level of segregation within this system is dependent on the degree of homology shared by chromosomes contributed by separate parents, it may be expected that different intraspecific combinations will segregate for the same or similar characteristics at different rates. Thus, intraspecific differences involving chromosome pairing affinities may be detectable within such a system even though morphological differences between sporophytes are not evident. In that *C. thalictroides* has been described as a polymorphic species (*10*), the ability to differentiate between individuals on the basis of chromosome pairing affinities may be an important factor to be considered in future taxonomic treatments of the genus.

The previous demonstration of homoeologous pairing and restricted segregation in duplex heterozygote *Ceratopteris* sporophytes (*3*) involved a diploid form of the genus ($n = 39$). Obviously, although this is the lowest extant number in the genus, the diploid form is, in fact, of polyploid derivation and tetraploid for those loci that have been shown to be maintained in a duplicated state. The present example dealing with *C. thalictroides* involves the tetraploid form of the genus ($n = 78$). Evolutionarily, it must represent an ancient octaploid. However, the inheritance pattern is identical to the model developed from the extant diploid form. Therefore, in spite of a major difference in ploidy level, both forms contain at least one locus present in a tetrasomic state.

L. G. HICKOK*

Department of Botany,
Mississippi State University,
Mississippi State 39762

References and Notes

1. E. J. Klekowski and H. G. Baker, *Science* **153**, 305 (1966); E. J. Klekowski, *Ann. Mon. Bot. Gard.* **59**, 138 (1972).
2. E. J. Klekowski, *Am. J. Bot.* **60**, 535 (1973).
3. L. G. Hickok, *ibid.* **65**, 516 (1978).
4. E. J. Klekowski and L. G. Hickok, *ibid.* **61**, 422 (1974).
5. D. W. Bierhorst, *ibid.* **62**, 448 (1975).
6. L. G. Hickok, *ibid.* **64**, 552 (1977).
7. The model is based on the observation that, if chromosome pairing within a bivalent forming duplex heterozygote is restricted to homologous chromosomes (A1/A1, a2/a2), no segregation can occur (0 percent *aa*); but if pairing is restricted to homoeologous pairs (A1/a2, A1/a2) segregation will be at a maximum of 25 percent *aa*. Thus, intermediate levels of segregation can be interpreted on the basis of intermediate levels of homologous and homoeologous pairing. Since one-quarter of the meiotic products are double recessives when pairing is 100 percent homoeologous, the level of homoeologous pairing can be calculated from the segregation frequency by multiplying by a factor of 4. Multivalent forming individuals must be treated somewhat differently because of the possibility of double reduction. A detailed description of the model is given by Hickok (*3*).
8. Stock 230Xn was derived from a spore collection obtained from a Malaysian plant growing in the Botanic Garden at the University of Malaya. Stock 205I was obtained from a spore collection taken from a plant growing at the Royal Botanic Gardens, Kew, England. The wild source of this plant is unknown. General culture methods utilized to establish homozygous sporophytes by selfing isolated gametophytes have been described by L. G. Hickok and E. J. Klekowski [*Am. J. Bot.* **60**, 153 (1973)].
9. Spore collections that had been surface-sterilized by treatment with dilute sodium hypochlorite [M. Schedlbauer and E. J. Klekowski, *Bot. J. Linn. Soc.* **65**, 399 (1972)] were sown on standard mineral nutrient medium solidified with 1 percent agar and contained within sterile disposable petri dishes (100 by 15 mm) (*8*). Subsequent pale gametophytes that developed were isolated into 250-ml flasks containing the same medium supplemented with 2 percent sucrose. All procedures were carried out in a laminar flow hood.
10. R. M. Lloyd, *Brittonia* **26**, 139 (1974).
11. I thank J. Wiseman for interest and technical assistance. Supported by NSF grants DEB76-09756 and DEB78-02809.
* Present address: Department of Botany, University of Tennessee, Knoxville 37916.

22 June 1978

Long Ascending Projections from Substantia Gelatinosa Rolandi and the Subjacent Dorsal Horn in the Rat

Abstract. *Small neurons of the substantia gelatinosa Rolandi and the subjacent dorsal horn of the spinal cord have been thought to exert a direct modulatory effect only on neurons located within a distance of a few spinal segments. By using the technique of retrograde transport of horseradish peroxidase, however, it has been found that in the rat a significant number of these cells, particularly those of the subjacent dorsal horn, ascend many spinal segments to the lateral cervical nucleus and to the lower brainstem. These data provide an anatomic basis for a role of substantia gelatinosa Rolandi and subjacent dorsal horn cells in modulating or contributing to sensory information transmission not only in nearby segments but in far distant structures.*

Cells of the substantia gelatinosa Rolandi [SGR; Rexed's lamina II in the cat (*1*)] and of the subjacent dorsal horn (SDH; corresponding approximately to Rexed's lamina III) make numerous synaptic contacts with primary afferent fibers and with the dendrites of larger neurons entering these areas from above and below (*2-7*). The axons of these larger neurons, in turn, make segmental and propriospinal connections (*8*), and many are now known to ascend for long distances before synapsing in the brainstem and thalamus (*9*). Although direct physiological evidence is lacking, the connections formed by the small SGR and SDH cells have long been thought to qualify them for an important role in the modulation of reflexes and of information transmission to the brain (*4, 6, 7, 10*). Szentágothai (*4*) reported that lesions within laminae II and III of the cat dorsal horn produced degeneration at a distance of not more than three spinal segments. Consequently, the area directly affected by the modulating influences of SGR and SDH cells has been thought to be quite restricted. However, in the course of studying the cells of origin of the spinocervical tract in the rat, we have seen and now report that in this species many SGR and SDH cells possess far longer projections.

Adult male Sprague-Dawley rats were deeply anesthetized with sodium pentobarbital, and a laminectomy was per-

0036-8075/78/1201-0984$00.50/0

formed over segments C1 to C3. In six rats a recording micropipette filled with a 2 to 5 percent solution of horseradish peroxidase (HRP) mixed in 5 percent KCl-tris buffer was inserted under microscopic control through the C2 dorsal roots into the dorsolateral funiculus. After a physiologically identified neuron was isolated in the lateral cervical nucleus (LCN) (*11*), HRP was iontophoretically ejected from the pipette tip (*12*). In an additional ten animals small pressure injections of HRP (0.05 μl, 40 percent) were made at various levels of the brainstem and thalamus. After a recovery period of 48 to 72 hours, rats were anesthetized again and perfused through the heart with buffered saline followed by a solution 1 percent in paraformaldehyde and 3 percent in glutaraldehyde. Coronal and sagittal sections (50 μm) were reacted for the presence of HRP, using the *o*-dianisidine protocol of deOlmos (*13*). Adjacent sections were stained for Nissl material with thionine, for myelin with osmium tetroxide, and for both by the method of Klüver and Barrera (*14*).

To prevent possible deleterious effects of water on the *o*-dianisidine reaction product, we used noncounterstained material for microscopy and reconstruction in these studies. Nonetheless, by a combination of bright- and dark-field microscopy, the dorsal portion of the spinal gray matter could be readily divided into three distinct regions (Fig. 1A). Most superficially, the dorsal horn is capped by a layer of material (indicated by arrowheads in Fig. 1A) that is granulated in appearance when compared to the gray matter bordering it ventrally. In Nissl sections, this band is seen to be composed of large and small cells with somata oriented transversely and rostrocaudally. This area is the marginal zone, or Rexed's lamina I in the cat (*1*). Immediately ventral, high-interference microscopy in unstained tissue reveals a translucent band (40 to 85 μm dorsoventrally) running across the dorsal horn. In adjacent stained sections, this band is seen to be virtually devoid of myelin and to contain a large number of closely packed small, round, or oval cell bodies. This area is the substantia gelatinosa Rolandi, or in the cat, Rexed's lamina II (*1*). Immediately below the SGR lies an area (50 to 120 μm dorsoventrally) that in unstained tissue often has a frosted or highly stippled appearance. This area, referred to here as the SDH, is composed primarily of small oval cells and occasional larger cells and can be distinguished from the SGR by the presence of many myelinated axons. The precise correspondence between the SDH of the rat and Rexed's lamina III in the cat is difficult to assess, particularly since the criteria used by Rexed (*1*) to demarcate the border between laminae III and IV were not clearly stated.

The following procedure was employed for the majority of HRP-labeled neurons to ensure accuracy of reconstruction. Cells were photographed and carefully plotted in relation to landmarks within the gray matter, such as blood vessels. The tissue was then counterstained, and the area in which the labeled cells appeared was examined (*15*). The location of each cell in the counterstained tissue was the same as that determined in the unstained material.

Small injections of HRP into the LCN labeled several different cell types within the dorsal horn in all animals studied (*16*). As in the cat (*17*), such injections labeled medium to large neurons within the nucleus proprius immediately ventral to SDH. Unlike the results in the cat, however, labeling in the rat was occasionally seen in large Waldeyer cells of the marginal zone, whose dendrites are observed to extend across the dorsal horn in coronal sections. Also within the marginal zone, and to a lesser extent in the most dorsal portion of the SGR, labeling was seen in the small "limiting" cells of Cajal (*2*). These cells are easily distinguished from other small SGR neurons by their more dorsal position within the gray matter and, more importantly, by the characteristic transverse orientation of their dendrites. Within the SGR, injections of HRP into C2 labeled a small number of "central" cells (*18*) ipsilaterally as far caudally as T2. In the shape and size of their somata and in their proximal dendritic configurations, these cells strongly resemble the SGR neurons described in numerous Golgi investigations (*2–4, 6*). On the other hand, HRP injections labeled a far greater number of central cells in the SDH. Such cells were seen most frequently in the cervical enlargement (20 to 25 cells per longitudinal millimeter of cord sampled). Labeling was greatly reduced caudal to T2, with only occasional and widely scattered central cells being found.

Subsequent experiments revealed that long projections from central cells of the SGR and SDH are not limited to spinal destinations. In one rat, for example, an HRP injection that was made laterally in the region of the pontine-medullary junction (*19*) labeled central cells within the SGR as far caudally as C8 and within the SDH as far caudally as T12 (Fig. 1, B to F). Although this and similar injections

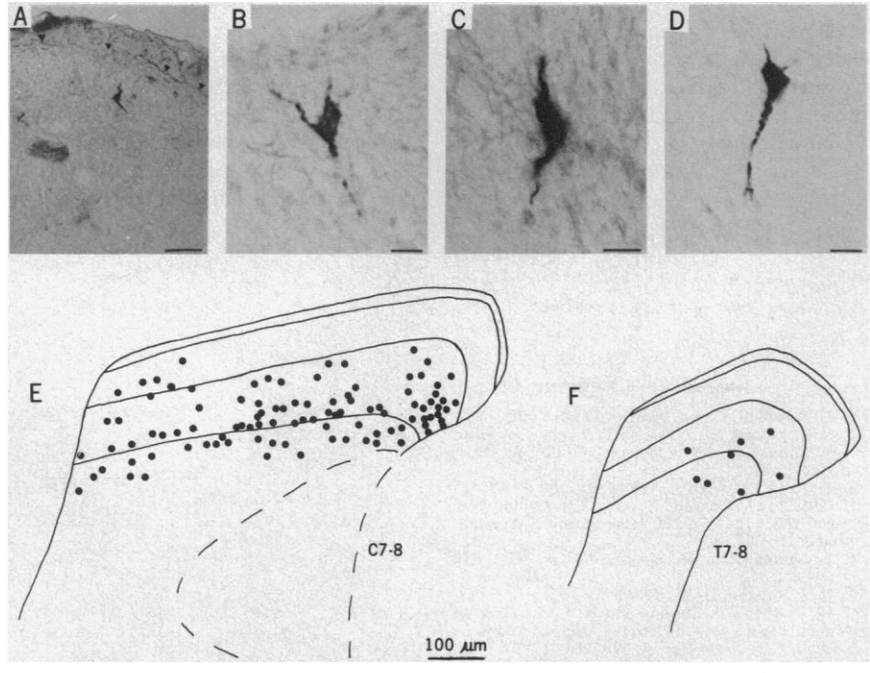

Fig. 1. Central cells of the dorsal horn (*18*) labeled by an injection of HRP into the lateral brainstem at the pontine-medullary junction (*19*). (A) Unstained tissue showing the major subdivisions of the dorsal portion of the spinal gray matter. Arrowheads indicate the dorsal border of the marginal zone, below which the SGR is seen as a translucent band extending across the dorsal horn. The SDH appears as a stippled area bordering the SGR ventrally. A relatively large, noncentral cell can be seen in the SDH with a dentrite extending into the SGR (scale bar, 50 μm). (B to D) Individual central cells within segments C7 and C8 from the SGR, dorsal SDH, and ventral SDH, respectively (scale bars, 10 μm). (E) Reconstruction of central cells encountered within three longitudinal millimeters of tissue from segments C7 and C8. (F) Reconstruction of central cells from 3.5 mm of tissue from T7 and T8.

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.

GLENN J. GIESLER, JR.*
J. TIMOTHY CANNON
GIDEON URCA†
JOHN C. LIEBESKIND
Department of Psychology, University of California, Los Angeles 90024

References and Notes

1. B. Rexed, *J. Comp. Neurol.* **96**, 415 (1952).
2. S. R. y Cajal, *Histologie du Système Nerveux de l'Homme et des Vertebres* (Maloine, Paris, 1909), vol. 1.
3. A. A. Pearson, *Arch. Neurol. Psychiatry* **68**, 515 (1952); J. A. Beal and C. A. Fox, *J. Comp. Neurol.* **168**, 113 (1976); H. Mannen and Y. Sugiura, *ibid.*, p. 303.
4. J. Szentágothai, *J. Comp. Neurol.* **122**, 219 (1964).
5. H. J. Ralston, III, *Z. Zellforsch. Mikrosk. Anat.* **67**, 1 (1965); P. Sterling and H. G. J. M. Kuypers, *Brain Res.* **4**, 1 (1967); L. Heimer and P. D. Wall, *Exp. Brain Res.* **6**, 89 (1968).
6. M. E. Scheibel and A. B. Scheibel, *Brain Res.* **9**, 32 (1968).
7. M. Réthelyi and J. Szentágothai, *Exp. Brain. Res.* **7**, 258 (1969).
8. A. G. Brown, C. R. House, P. K. Rose, P. J. Snow, *J. Physiol. (London)* **260**, 719 (1976); H. Burton and A. D. Loewy, *Brain Res.* **116**, 485 (1976); J. Rastad, E. Jankowska, J. Westman, *ibid.* **135**, 1 (1977).
9. D. L. Trevino, J. D. Coulter, W. D. Willis, *J. Neurophysiol.* **36**, 750 (1973); D. Albe-Fessard, A. Levante, Y. Lamour, *Adv. Neurol.* **4**, 157 (1970); D. L. Trevino and E. Carstens, *Brain Res.* **98**, 177 (1975); G. J. Giesler, Jr., D. Menétrey, G. Guilbaud, J. M. Besson, *ibid.* **118**, 320 (1976).
10. P. D. Wall, *J. Physiol. (London)* **164**, 508 (1962); R. Melzack and P. D. Wall, *Science* **150**, 971 (1965); F. W. L. Kerr, *Exp. Neurol.* **16**, 359 (1966); D. Denny-Brown, E. J. Kirk, N. Yanagisawa, *J. Comp. Neurol.* **151**, 175 (1973); F. W. L. Kerr, *Mayo Clin. Proc.* **50**, 685 (1975).
11. G. Urca, G. J. Giesler, Jr., J. C. Liebeskind, *Neurosci. Abstr.* **3**, 508 (1977).
12. A positive current of 2.5 μA for 5 minutes, followed by 5 μA for 5 minutes, then 10 μA for 10 to 15 minutes, was used to eject HRP from pipette tips of 1.0 to 1.5 μm.
13. J. deOlmos, *Exp. Brain Res.* **29**, 541 (1977).
14. H. Klüver and E. Barrera, *J. Neuropathol. Exp. Neurol.* **12**, 400 (1953).
15. In fact, we observed that counterstaining did not significantly alter the reaction product in many labeled cells. Further affirmation of the accuracy of our reconstructions was provided by this material.
16. Several features of these experiments minimize the possibility that labeling following LCN injections resulted from uptake through damaged nonterminating axons. First, injections were made through electrodes with small tips after a single penetration to minimize mechanical damage to the area. Second, areas (such as the nucleus raphe magnus) known to contribute fibers to the dorsolateral funiculus that do not terminate in the LCN were not labeled in these studies.
17. A. D. Craig, Jr., *Neurosci. Lett.* **3**, 173 (1976).
18. Central cells are considered to be those having somata measuring 9 by 14 μm or less and having a dorsoventral dendritic orientation.
19. This injection produced a dark green acellular reaction product in the cochlear nucleus, the tract and main sensory nucleus of V, the lateral lemniscus, lateral portions of the cerebellar cortex, and the cerebellar roof nuclei. The acellular area produced by an HRP injection in the visual system has been shown [H. Vanagas, H. Hollander, H. Distel, *J. Comp. Neurol.* **177**, 193 (1978)] to be the area from which retrograde transport occurs.
20. W. D. Willis, R. B. Leonard, D. R. Kenshalo, Jr., *Science* **202**, 986 (1978).
21. T. Fukushima, J. D. Grabow, F. W. L. Kerr, *Neurosci. Abstr.* **3**, 481 (1977).
22. Supported by NIH grant NS 07628. J.T.C. was supported by PHS research fellowship 1 F32 NS 05702.
* Present address: Marine Biomedical Institute, 200 University Boulevard, Galveston, Texas 77550.
† Present address: Department of Physiology and Pharmacology, Tel Aviv University, Ramat Aviv, Israel.

8 August 1978

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-

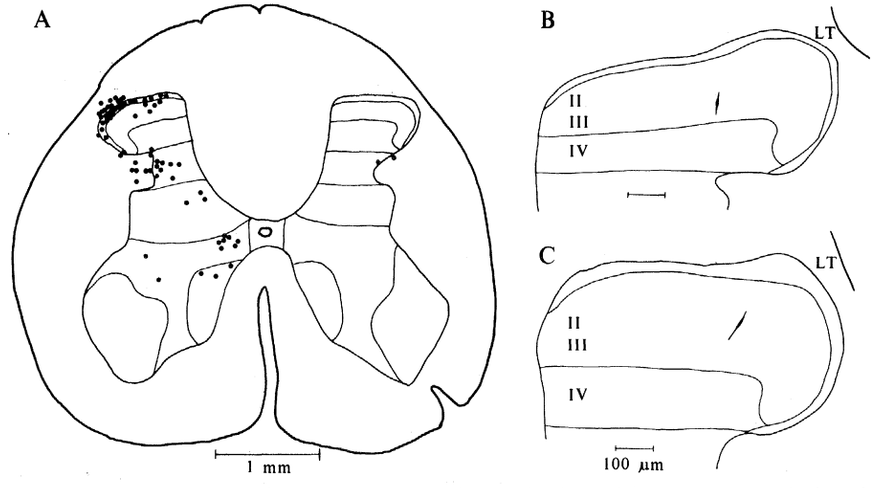

Fig. 1. (A) Distribution of spinothalamic tract cells in the lumbar enlargement of the spinal cord of a monkey. The cells were labeled by an injection of HRP into the region of the ventral posterior nucleus of the thalamus. Most of the cells were contralateral to the injection site. The cells shown were 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.)

0036-8075/78/1201-0986$00.50/0