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Afferent Input to Nucleus Submedius in Rats: Retrograde Labeling of Neurons in the Spinal Cord and Caudal Medulla

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In cats, spinal and medullary input to the thalamic nucleus submedius (Sm) arises almost exclusively from neurons in the marginal zone. As a result, it has been proposed that Sm may be specifically involved in nociception. In the present study, we determined the locations of neurons in the spinal cord and caudal medulla that project to Sm in rats. Iontophoretic injections of Fluoro-Gold or pressure injections of Fast blue were made into Sm. In each of the 6 rats that received small injections of Fluoro-Gold into Sm, only a small number (mean = 90) of retrogradely labeled neurons were found throughout the 18 segments of the spinal cord examined. Surprisingly, almost no labeled neurons (<1%) were counted in the marginal zone of the spinal cord. The majority were located in the deep dorsal horn and intermediate zone/ventral horn. In contrast, many neurons were labeled in the marginal zone of nucleus caudalis. Injections of Fluoro-Gold into any of a number of nuclei near Sm also labeled only a small number of neurons in the spinal cord and almost no neurons in the marginal zone. Using identical injection parameters, we injected Fluoro-Gold into the ventrobasal complex or posterior thalamic group. Hundreds of neurons in the spinal cord, including many in the marginal zone, were labeled following these injections. These results indicate that the techniques used to inject Fluoro-Gold into Sm were capable of labeling many projection neurons, including those in the marginal zone. Larger pressure injections of Fast blue were also made into Sm of 3 rats. The distribution of labeled neurons in nucleus caudalis and the spinal cord was similar to that following iontophoretic injections of Fluoro-Gold. Again, few marginal zone neurons were labeled in the spinal cord in any of these rats. Therefore, our results indicate that few spinothalamic tract neurons appear to project to Sm or any of several adjacent nuclei, and virtually no marginal zone neurons in the spinal cord project to these areas.

Several anterograde tracing studies in rats, cats, and monkeys have shown that neurons in the spinal cord (Craig and Burton, 1981; Mantyh, 1983a, b; Peschanski et al., 1983; Robertson et al., 1983; Craig and Burton, 1985) and spinal trigeminal nucleus (Craig and Burton, 1981; Peschanski, 1984; Fig. 33 by L. Kruger, J. L. Krettek, R. F. Young, and J. Curtis in Albe-Fessard et al., 1985; Ma et al., 1988) project to the dorsal portion of the thalamic nucleus submedius (Sm). The input is topographically organized; the projection from the spinal cord is to the rostral half of Sm, and the projection from the spinal trigeminal nuclei terminates in the caudal half of the nucleus (Craig and Burton, 1981). Retrograde tracing studies using HRP suggest that in cats, the input to Sm arises almost exclusively from neurons in the marginal zone of the spinal and medullary dorsal horns (Craig and Burton, 1981). Based on these anatomical data and the fact that many neurons in the marginal zone respond specifically to noxious stimuli (Christensen and Perl, 1970; Kumazawa et al., 1975; Price et al., 1976), Craig and Burton (1981) proposed that Sm may have an important role in nociception.

Recent electrophysiological and anatomical studies in rats support this hypothesis. Neurons have been recorded in Sm that respond specifically to noxious mechanical and thermal stimuli (Dostrovsky and Guilbaud, 1988; Miletic and Coffield, 1989). Others respond to innocuous mechanical stimuli, but respond at higher frequencies to noxious stimulation (Miletic and Coffield, 1989). Many nociceptive neurons in Sm have large bilateral receptive fields (Dostrovsky and Guilbaud, 1988; Miletic and Coffield, 1989), suggesting that Sm is not likely to play a role in sensory-discriminative aspects of nociception. Also, noxious mechanical stimulation of the hindlimb induces expression of c-*fos*-like protein in a number of neurons within Sm in rats (Bullitt, 1989). Expression of c-*fos* is thought to be an anatomical marker for increased activity in neurons (Hunt et al., 1987). Anatomical studies in rats (Jones and Leavitt, 1974; Krettek and Price, 1977; Herkenham, 1979; Ma et al., 1988) and cats (Craig et al., 1982) have demonstrated that neurons in Sm project to the ventrolateral orbital cortex, suggesting that Sm may convey nociceptive information to the cortex (Craig et al., 1982).

The locations of neurons projecting to Sm in species other than the cat have not been investigated thoroughly. Since many behavioral, pharmacological, and physiological studies of pain have been done in rats, we thought it would be valuable to determine the inputs to Sm in this species. In the present study, the sources of afferent input to Sm from the spinal cord and caudal medulla were examined following small injections of sensitive fluorescent retrograde tracers into Sm of rats.

Materials and Methods

Male Sprague-Dawley rats (320–570 gm) were anesthetized with sodium pentobarbital and placed in a stereotaxic frame. In each of 17 rats, attempts were made to inject Fluoro-Gold (FG; 1% solution dissolved in 0.1 M NaCl) iontophoretically into Sm. Iontophoretic injections were

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chosen because of the ability to restrict the size and spread of the injections (Schmued and Fallon, 1986; Pieribone and Aston-Jones, 1988). Glass micropipettes with tip diameters of approximately 10 μm were used. In each case, Fluoro-Gold was iontophoretically injected using approximately 48 μA-min of anodal current. Injections were made in approximately the anterior–posterior center of Sm to enable spread of the injection into both the spinal and trigeminal zones of input. In each of 2 additional rats, an injection was made at 2 different anterior–posterior coordinates to insure that the entire rostrocaudal extent of Sm was filled with the tracer.

Following a 3–5 d survival period (Burstein et al., 1990a, b), rats were deeply anesthetized with sodium pentobarbital and perfused with 0.9% saline followed by 10% formalin. The brain, including caudal medulla, and 18 spinal cord segments (C1-2, C4-5, C7-8, T4, T6, T8, T12, L1-3, L4-5, L6-S2) were identified and removed. The diencephalon from each rat was cut transversely on a freezing microtome at 100 μm immediately following dissection. Identified spinal cord segments and caudal medulla were placed in 10% formalin for at least 12 hr and subsequently sectioned at a thickness of 40 μm. Alternate serial sections were mounted and sealed under a coverslip with DPX. Sections were examined microscopically using reflected ultraviolet illumination. Injection sites were initially reconstructed using dark-field microscopy. To confirm the location and boundaries of nucleus submedius, the tissue was subsequently counterstained with cresyl violet or Neutral red and reexamined using bright-field microscopy. Injection sites and retrogradely labeled neurons in the caudal medulla and the spinal cord were reconstructed with the aid of a camera lucida drawing attachment.

Iontophoretic injections of Fast blue were attempted but were not successful. The tips of the micropipettes evidently became plugged, and subsequently, it was difficult to maintain the current necessary to inject adequate amounts of the tracer. Therefore, in each of 3 rats, a pressure injection (100–200 nl) of a 1% solution of Fast blue dissolved in 0.1 M NaCl was made into Sm. Injections were made using a micropipette attached to the end of a Hamilton microsyringe. One rat was allowed to survive 4 d; the other 2 were allowed 11 d because it has been suggested that longer survival periods are required for transport of Fast blue to the lumbosacral cord (Craig et al., 1989b). The same areas of brain and spinal cord were processed.

Labeled neurons in the spinal cord were classified according to their location in 1 of 5 easily recognized areas: superficial dorsal horn, deep dorsal horn, intermediate zone/ventral horn, area around the central canal, lateral spinal nucleus/lateral cervical nucleus (Burstein et al., 1990a). Within the caudal medulla, labeled neurons in the trigeminal nucleus caudalis were classified as either superficial or deep. Neurons were classified as superficial if they were located in the marginal zone, substantia gelatinosa, or the overlying spinal trigeminal tract. Neurons were classified as deep if they were located in an area extending approximately 800 μm ventromedially from the ventral border of the substantia gelatinosa. Nucleus caudalis was considered to extend from the pyramidal decussation to the level of the obex (Torvik, 1956).

To evaluate the effectiveness of our techniques, the ventrobasal complex (VBC) and/or posterior thalamic group (Po) was injected in six additional rats. In each of these animals, iontophoretic injections of FG were made using injection currents similar to those used to inject Sm. Survival times were also the same. Injections were made into VBC in 3 rats (VBC1–3) at approximately the same anterior–posterior level as those into nucleus submedius. A second group of 3 rats received single injections near the border between Po and VBC (Po/VBC1–3).

Results

Injection sites of FG were generally spherical and consisted of a dense core of the tracer in which considerable necrosis was evident. This core was surrounded by a diffuse halo of the tracer. No spread of FG dorsally along the micropipette track could be seen following any of the iontophoretic injections (Figs. 1*A*; 2*A*; 3, *A–E*; 4, *A–D*; 5*A*; 6*A*).

Injections of FG into Sm

In each of 6 rats (Sm1–6), the necrotic core of the injection filled much of the dorsal half of Sm (the area that receives input from the spinal cord and spinal trigeminal nuclei). A photomicrograph of an injection (Sm1) into Sm is illustrated in Figure 1*A, B*. The core of this injection was restricted to Sm. The halo of FG extended into parts of the rhomboid nucleus, nucleus reuniens, and the anteromedial nucleus. The mediolateral diameter of the core of the injection was approximately 525 μm. The diameter of the injection, including the halo, was approximately 1.2 mm. The entire anterior–posterior extent of Sm was included in the injection (in rats, Sm extends approximately 1.0–1.5 mm rostrocaudally; Craig and Burton, 1981; Paxinos and Watson, 1986). The injection extended slightly beyond (100–200 μm) the anterior limit of Sm. The distribution of neurons that were labeled by this injection in the caudal medulla and spinal cord is illustrated in Figure 2.

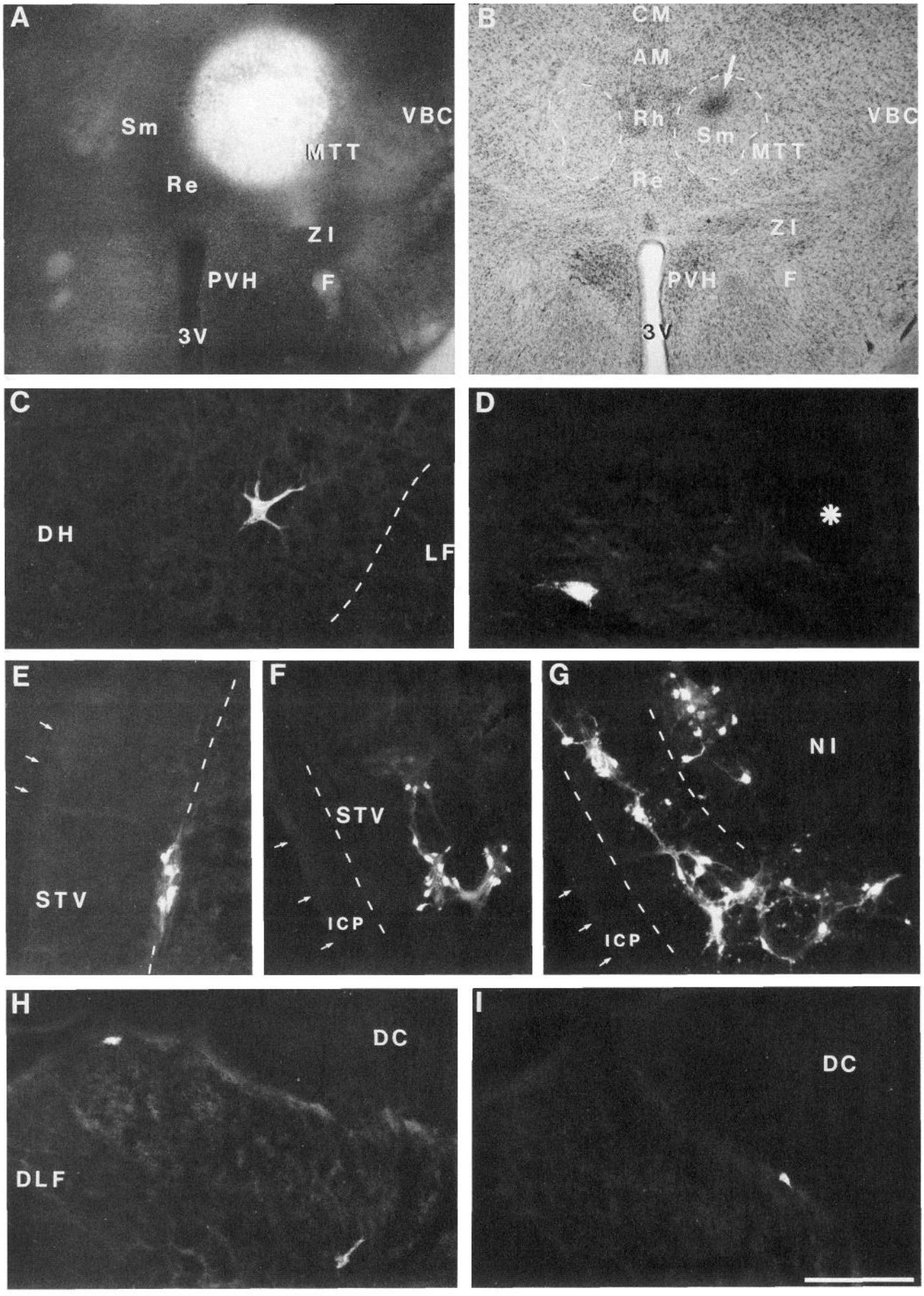
Seventy-nine labeled neurons were found bilaterally in the caudal medulla (Fig. 2*B*). Most were located in the marginal zone of rostral nucleus caudalis. A few were seen in deeper areas of nucleus caudalis. Labeled neurons were also scattered sparsely throughout the reticular formation and the dorsal column nuclei.

A total of 72 retrogradely labeled neurons was found bilaterally throughout the 18 examined spinal cord segments (Fig. 2*C*). The majority were found in the deep dorsal horn and intermediate zone/ventral horn. A few were seen in the gray matter around the central canal, the lateral spinal nucleus, and the lateral cervical nucleus. None were seen in the marginal zone at any level of the spinal cord in this rat.

Reconstructions of the injection sites from cases Sm2–6 are shown in Figure 3, *A–E*, respectively. In each of these animals, the necrotic core of the injection included much of the dorsal half of Sm. The mediolateral diameters of the cores of the injections ranged from approximately 500 μm in Sm3 (Fig. 3*B*) to 750 μm in Sm2 (Fig. 3*A*). The diameters of the injections including the halo varied from approximately 975 μm in Sm3 (Fig. 3*B*) to 1.4 mm in Sm2 (Fig. 3*A*). In each case, the halo of the injection extended to the anterior limit of Sm and in several rats, the halo spread 100–400 μm anterior to Sm. The halo of FG extended to the posterior limit of Sm in 4 of the 6 rats. In the other 2 rats, the halo of FG extended to within approximately 100–200 μm of the caudal limit of Sm.

Following each injection into nucleus submedius (Sm1–6), labeled neurons were seen bilaterally in the caudal medulla. A mean number of approximately 80 neurons was counted in nucleus caudalis in each of the 6 rats. Approximately 81% of these were located in superficial nucleus caudalis (Fig. 1, *E, F*) and 19% in deep nucleus caudalis. Within the superficial nucleus caudalis, a distinctive rostrocaudal distribution of labeled neurons was evident in each rat. The highest concentrations occurred rostrally near the border with nucleus interpolaris (Fig. 2*B*). These neurons were usually found in clusters in the ventrolateral area of nucleus caudalis (Fig. 1*F*). About 15% of the labeled neurons counted in the caudal medulla were ipsilateral to the injection site in the 3 cases (Sm1–3) in which the tracer did not cross the midline. Many labeled neurons were seen in nucleus interpolaris (Fig. 1*G*). In each case, a few were also found in the medullary reticular formation and the dorsal column nuclei.

The total number of labeled neurons counted in the 18 examined spinal cord segments in cases Sm2, 3, 4, and 5 was 108, 1, 96, and 35, respectively. In case Sm6, an injection was made at 2 different anterior–posterior coordinates (Fig. 3*E*). The necrotic core of the anterior injection was well restricted to Sm. However, the posterior injection was centered dorsal to Sm in the central medial nucleus. The largest number of labeled neu-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rons (230) in the spinal cord was found in this rat, but none were seen in the marginal zone.

The mean number of labeled neurons in the spinal cords of rats Sm1–6 was approximately 90 neurons (Table 1).[1] More than 80% were located either in the deep dorsal horn (51%; Fig. 1*C*) or intermediate zone/ventral horn (30%). The area around the central canal contained 11% (Fig. 1*D*) and the lateral spinal nucleus/lateral cervical nucleus contained 7% of the labeled neurons. Fewer than 1% of the labeled neurons in the spinal cord were in the superficial dorsal horn. In the 6 rats, a total of 4 neurons was labeled in the superficial dorsal horn. Each of these 4 neurons was in the marginal zone; none were in substantia gelatinosa. The largest number in the superficial dorsal horn in any single rat was 2 (Sm5). In 3 of the 6 cases (Sm4–6), the halo of FG crossed the midline. The number and distribution of labeled neurons in these rats did not appear to differ from those that received a unilateral injection, indicating that the FG that crossed the midline apparently did not label a large number of neurons.

The cervical segments contained approximately 74% of the retrogradely labeled neurons, 10% were in the thoracic segments, and 16% were in the lumbosacral segments. Approximately 64% of the labeled neurons in the spinal cord were found contralateral to the injection site in the 3 cases (Sm1–3) in which the tracer did not cross the midline.

Injections of FG into areas near Sm

In 13 rats, the necrotic core of the injection missed the dorsal half of Sm (NonSm1–13; Fig. 4, *A–D*). These injections were approximately the same size as those into Sm and were located in one or more of the following structures: rhomboid nucleus, nucleus reuniens, ventral nucleus reuniens, zona incerta, central medial nucleus, paracentral nucleus, ventral lateral nucleus, ventral medial nucleus, anterior medial nucleus, prerubral field, an unnamed area in the ventromedial thalamus (ventral to nucleus reuniens, and dorsal to the third ventricle) or the mammillothalamic tract (Paxinos and Watson, 1986). These injections surrounded Sm anteriorly, posteriorly, dorsally, ventrally, medially, and laterally. The results of these injections are included for 2 main reasons. First, since our data indicate that neurons in the marginal zone of the spinal cord do not appear to project to Sm, it is possible that they may project to an area near Sm. These injections should reveal the presence of such a projection if it exists. Second, little is known about the cells of origin of spinothalamic tract (STT) projections to these small thalamic areas.

Each of these injections retrogradely labeled neurons in the spinal cord. The total number of neurons counted in the spinal cord of each rat is presented in Table 2. The number and distribution of labeled neurons was similar to that following injections into Sm. Over two-thirds were located in either the deep dorsal horn (39%) or intermediate zone/ventral horn (29%). About 3% were located in the superficial dorsal horn. Nearly half (17/39) of the labeled neurons in the superficial dorsal horn in these cases were found in one rat (NonSm4). Approximately 62% were counted in cervical cord, 10% in thoracic cord, and 28% in lumbosacral cord.

Neurons in the caudal medulla were also labeled by injections into areas near Sm (Table 2). In 5 (NonSm3, 7, 9, 10, 13) of the 13 animals, labeled neurons were seen primarily in the superficial nucleus caudalis in a pattern similar to that seen following injections into Sm. A mean number of approximately 45 neurons was counted in nucleus caudalis in these 5 animals. However, the locations of the injections in these cases (Fig. 4, *B–D*) revealed no obvious similarities to explain the pattern of labeled neurons. In the other 8 cases, fewer neurons were labeled (approximately 10 in each case) within nucleus caudalis.

Injections of FG into ventrobasal complex or posterior thalamic group

Our injections into or near Sm consistently labeled a relatively small number of neurons in the spinal cord. To determine whether the techniques we used were capable of labeling large numbers of neurons, iontophoretic injections of FG were made into the VBC or Po of 6 rats. These areas are known to receive a dense projection from the spinothalamic tract (Lund and Webster, 1967; Mehler, 1969; Zemlan et al., 1978; Peschanski et al., 1983; Ma et al., 1986). The injection parameters were identical to those used to inject Sm.

In 3 rats, the injection sites were located in anterior VBC at approximately the same anterior–posterior level as the injections into Sm. The diameters of the necrotic cores of the injections ranged from approximately 470 to 885 μm. The diameters of the injections including the halo varied from 1.0 to 1.6 mm. Therefore, the sizes of the injection sites were similar to those of injections into Sm.

An example of an injection into the anterior VBC is illustrated in Figure 5*A*. Many neurons were retrogradely labeled in the caudal medulla (379) and spinal cord (607) following this injection (Fig. 5, *B, C*). Labeled neurons within the spinal cord were concentrated in the deep dorsal horn of C1-2 and the lumbar cord.

In each of the 3 rats that received injections into VBC, a large

[1] The mean of 90 labeled neurons was based on all 6 cases. As mentioned previously, the injections in Sm6 included not only Sm, but also spread into other nuclei, including the central medial nucleus (Fig. 3*E*). In contrast, cases Sm1–5 received single injections that were primarily restricted to Sm. The mean number of labeled neurons in the 18 spinal segments in Sm1–5 was 62 ± 44.

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Figure 1. Photomicrographs of an iontophoretic injection of FG into Sm and labeled neurons in the spinal cord and caudal medulla. *A,* Darkfield photomicrograph of an injection of FG into Sm (Sm1). *B,* Bright-field photomicrograph of the same section after counterstaining with cresyl violet. *Arrow* indicates the core of the injection, and *dashed line* indicates the approximate outline of Sm. *C–G,* FG-labeled neurons following injections into Sm. *C,* Ipsilateral deep dorsal horn of C1-2. *Dashed line* represents the border between the lateral reticulated area of *DH* and *LF. D,* Contralateral area around the central canal of C7-8. *Asterisk* indicates the location of the central canal. *E,* Contralateral superficial nucleus caudalis. *Dashed line* indicates the border between nucleus caudalis and *STV.* In *E–G, arrows* indicate the lateral edge of the brainstem. *F,* Contralateral superficial nucleus caudalis near the border with nucleus interpolaris. These labeled neurons were located in the ventrolateral area of nucleus caudalis just medial to the *STV. Dashed line* indicates the border between *STV* and *ICP. G,* Contralateral *NI.* Labeled neurons were located ventrolaterally both within *NI* and the spinal trigeminal tract, which is the area between the *dashed lines. H* and *I,* FG-labeled neurons following an injection near the border between the VBC and Po. *H,* Contralateral superficial dorsal horn and deep dorsal horn of C7-8. *I,* Contralateral superficial dorsal horn of C4-5. For abbreviations, see the Appendix. Scale bar, 1 mm for *A* and *B*; 0.2 mm for *C* and *E–I*; 0.1 mm for *D.*

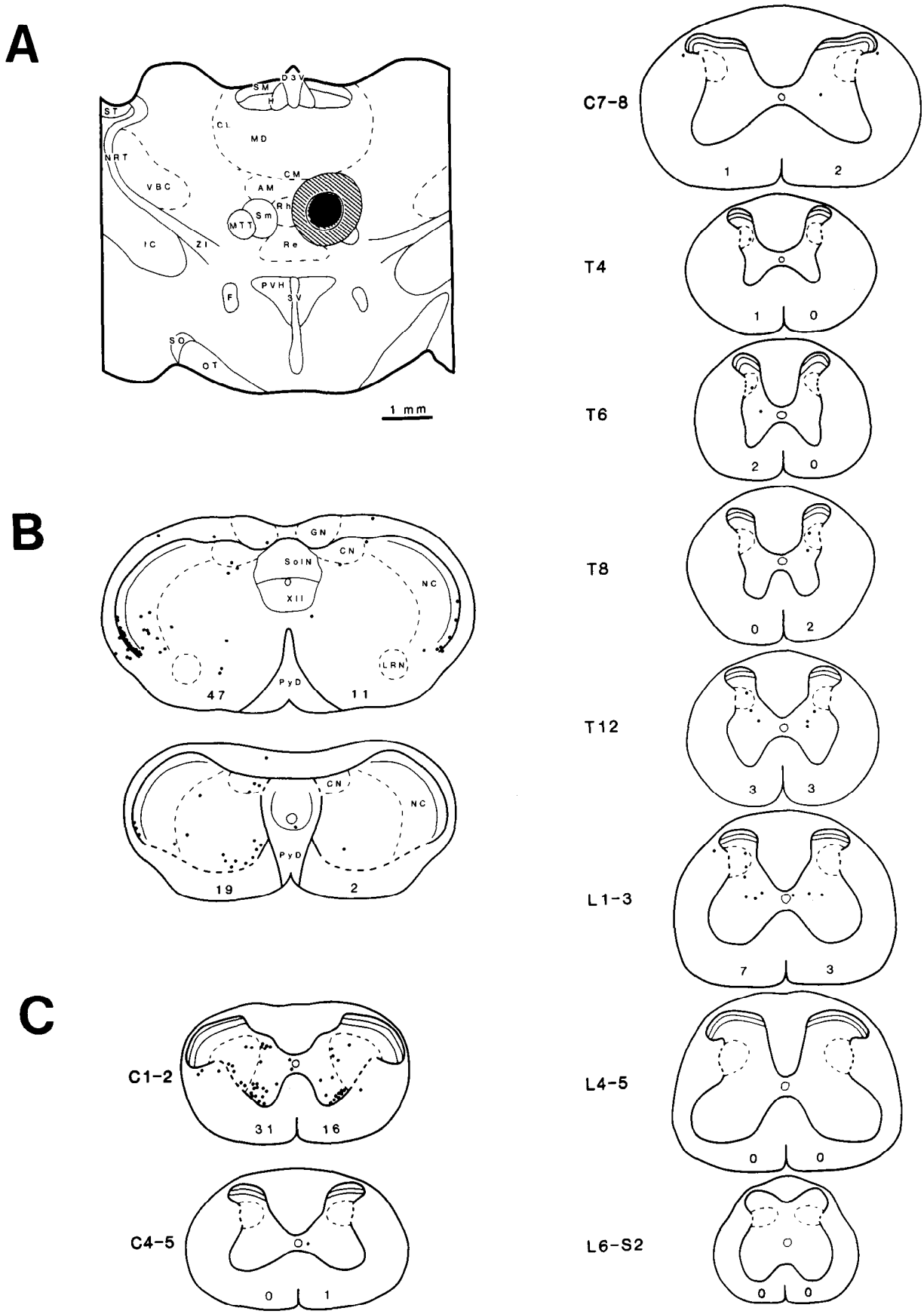

Figure 2. Reconstruction of an iontophoretic injection of FG into nucleus submedius and locations and numbers of retrogradely labeled neurons in alternate sections of the caudal medulla and 18 spinal cord segments (*Sm1*). *A*, The necrotic core of the injection was restricted to *Sm* and is represented by the black area. The halo of FG surrounding the core is shown by the *cross-hatched* area. The approximate location of *Sm* on the

number of labeled neurons was counted (419 ± 168). Retrogradely labeled neurons were found primarily in C1-2 (45%) and in the lumbar cord (52%; Fig. 5C).[2] Most labeled neurons were in the deep dorsal horn (74%) and intermediate zone/ventral horn (14%). Approximately 97% of the labeled neurons were located contralateral to the injection site. Retrogradely labeled neurons in the caudal medulla were restricted primarily to the contralateral dorsal column nuclei (Fig. 5B). In 2 of the animals, no labeling was present in either the superficial or deep nucleus caudalis. In the third case, 5 neurons were counted in the contralateral superficial nucleus caudalis.

In 3 rats (Po/VBC1–3), an area near the border between the posterior thalamic group and the caudal ventrobasal complex was injected iontophoretically with FG. In the best case (Po/VBC1; Fig. 6A), over 700 neurons were retrogradely labeled throughout the length of the spinal cord (Fig. 6C). Many neurons (122) were labeled in the marginal zone. Most were found throughout the cervical cord (Fig. 1, H, I); a few were also seen in lumbar segments (Fig. 6C).

A mean number of 387 ± 325 neurons was labeled in the three cases. Approximately 15% of the labeled neurons were located in the marginal zone. Fifty percent were found in the deep dorsal horn. Most of the labeling occurred in the cervical (82%) cord. Approximately 93% of the labeled neurons were found contralateral to the injection site. The distribution of labeled neurons in the caudal medulla differed from that following injections into Sm. A larger number of neurons in deep nucleus caudalis were labeled, and those in superficial nucleus caudalis were concentrated in the dorsal half of the nucleus (Fig. 6B). Labeled neurons were also present in the reticular formation and the dorsal column nuclei.

Injections of Fast blue into Sm

Craig et al. (1989a) reported that the fluorescent tracer Fast blue effectively labels small spinothalamic tract neurons in the marginal zone. Therefore, we made pressure injections of Fast blue into Sm of 3 rats (Sm7–9). One rat (Sm7) was allowed to survive 4 d; the other 2 (Sm8, 9) survived 11 d. An example of a pressure injection of Fast blue into Sm (Sm8) is illustrated in Figure 7A. The necrotic core of the injection was centered in the dorsal half of Sm. However, there was spread of the halo dorsally along the pipette track into several areas, including the central medial nucleus, the medial dorsal nucleus, the habenula, and the dorsal third ventricle. The injection spread approximately 2.0 mm rostrocaudally and included the entire anterior–posterior extent of Sm. The halo of the injection extended approximately 500 μm beyond the anterior limit of Sm and 100 μm beyond the posterior limit.

Following this pressure injection of Fast blue, labeled neurons were seen bilaterally in the caudal medulla. The locations and rostrocaudal distribution of labeled neurons in superficial nucleus caudalis were similar to that described following iontophoretic injections of FG into Sm. Many labeled neurons were located in superficial nucleus caudalis near the junction with nucleus interpolaris (Fig. 7B). In contrast, more labeled neurons were seen in the reticular formation after injections of Fast blue and some were also found in the nucleus of the solitary tract and the hypoglossal nucleus.

A total of 441 neurons was labeled throughout the length of the spinal cord in Sm8 (Fig. 7C; Table 3). The majority of labeled neurons were located in the deep dorsal horn (46%) and intermediate zone/ventral horn (28%). Three labeled neurons (<1%) were found in the marginal zone in this case. Labeled neurons were also found in the area around the central canal, the lateral spinal nucleus, and the lateral cervical nucleus. Approximately 56% of the labeled neurons in the spinal cord were found contralateral to the injection site.

The total number of neurons labeled in nucleus caudalis and the spinal cord for each of the 3 rats that received injections of Fast blue is summarized in Table 3. Over 70% of the labeled neurons in the spinal cord were located in the deep dorsal horn and intermediate zone/ventral horn. The area around the central canal and the lateral spinal nucleus/lateral cervical nucleus each contained approximately 13% of the labeled neurons. Less than 2% were located in the superficial dorsal horn. Approximately 62% of the labeled neurons were in the cervical cord, 10% were in the thoracic cord, and 28% were in the lumbosacral cord.

Discussion

Following small iontophoretic injections of FG into nucleus submedius of 6 rats, only a small number of retrogradely labeled neurons was observed in the spinal cord in each case. More than 80% of the neurons labeled were found in the deep dorsal horn or intermediate zone/ventral horn. Surprisingly, labeled neurons were virtually absent in the marginal zone throughout the length of the spinal cord.

The results of larger pressure injections of Fast blue into Sm of three rats confirmed those following small iontophoretic injections of FG. Again, many labeled neurons in the spinal trigeminal nucleus were found in superficial nucleus caudalis near the border with nucleus interpolaris. The distribution of labeled neurons in the spinal cord was also remarkably similar to that seen following injections of FG. Most of the labeled neurons were found in the deep dorsal horn and intermediate zone/ventral horn. Following injections of Fast blue, few neurons were seen in the marginal zone. In fact, in the 9 rats that received injections of either FG or Fast blue into Sm, a total of only 16 labeled neurons was counted in the marginal zone in over 5300 sections of spinal cord that were examined.

[2] Following each of these injections into the anterior VBC, many neurons were labeled in lumbar segments. Far fewer neurons were labeled in thoracic segments and the cervical enlargement (Fig. 5C). This organization is consistent with the known somatotopic input to the VBC from STT neurons; those in the lumbar cord project mainly to the anterior VBC, whereas those in the cervical cord project primarily to caudal VBC (Peschanski et al., 1983; Ma et al., 1986). However, in all cases, large numbers of neurons were also labeled in the rostral 2 cervical segments. The organization of the projections from these segments and the large, often whole-body receptive fields of many of the neurons in these segments (Carstens and Trevino, 1978) are not consistent with a simple somatotopic scheme. It should also be pointed out that each of our injections into the anterior VBC labeled many neurons in the contralateral cuneate nucleus (Fig. 5B), but failed to label neurons in the cervical enlargement (Fig. 5C). To our knowledge, this is the first indication in rats that the projections from the cuneate nucleus and STT neurons in the cervical enlargement may not overlap completely.

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right side is outlined. Note that in this and all subsequent figures, *dashed lines* represent approximate locations of boundaries and nuclei that were frequently difficult to determine specifically. Scale bar, 1 mm. *B*, Caudal medulla at level slightly posterior to obex (*top*) and approximately 1 mm posterior to obex (*bottom*). The *right side* is ipsilateral to the injection in all figures. *C*, Each labeled neuron in the spinal cord in alternate sections is indicated by a *dot*. The total number of labeled neurons found in each side of the indicated segments in alternate sections is shown.

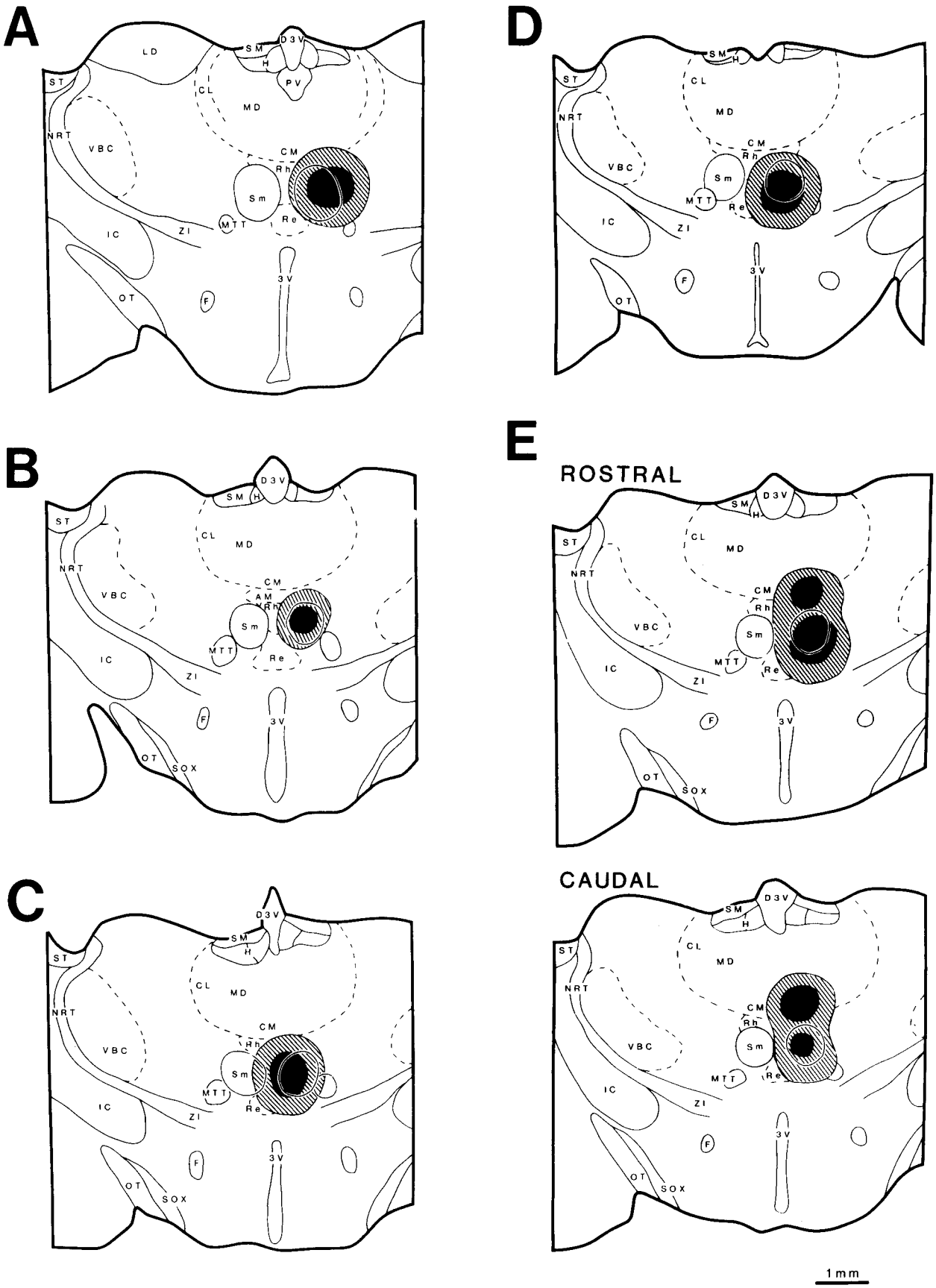

Figure 3. Drawings of injections of FG into nucleus submedius (*Sm2–6*). *A–E,* Sm2, 3, 4, 5, and 6, respectively. Scale bar, 1 mm.

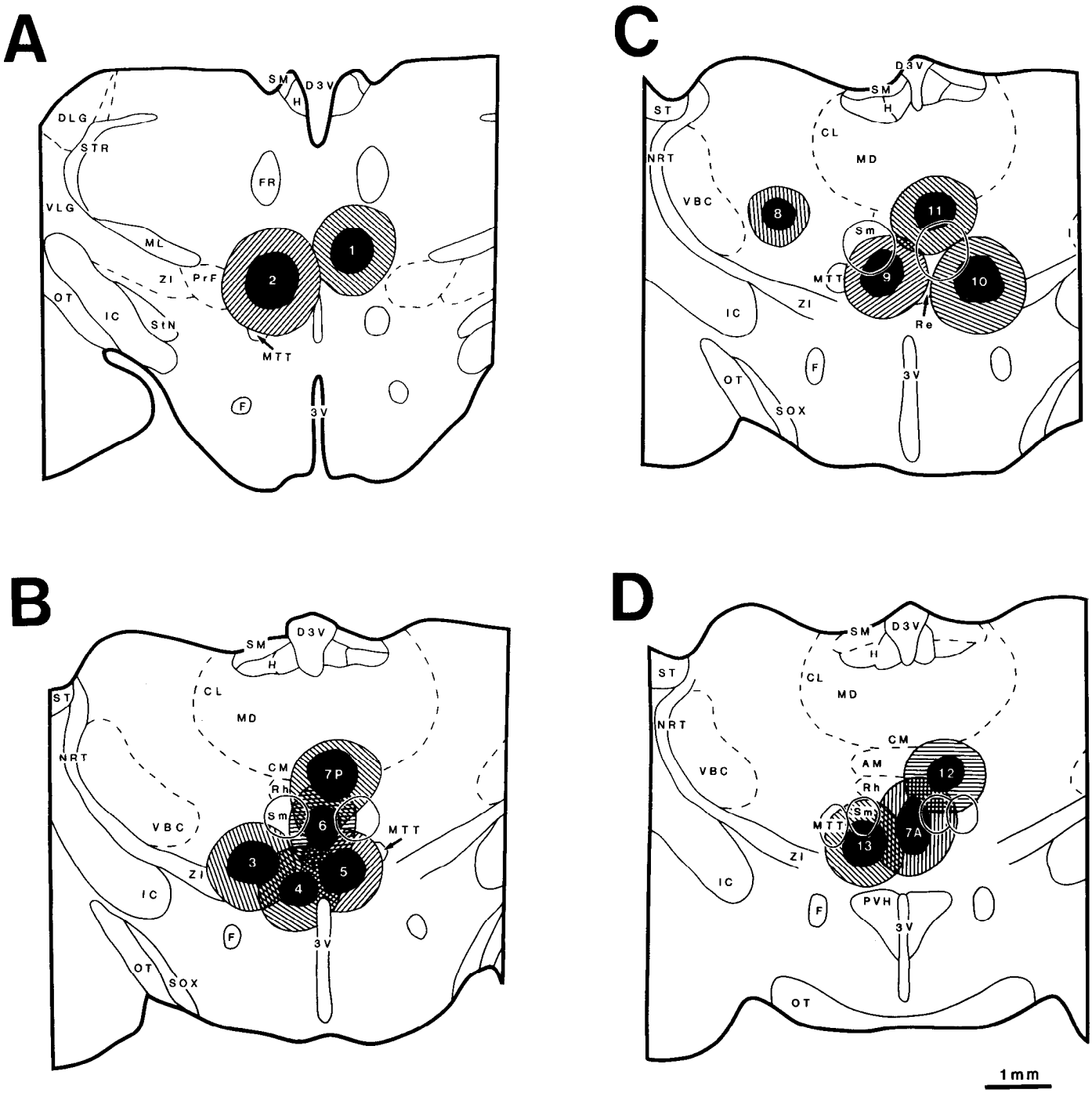

Figure 4. Drawings of injections of FG into areas near nucleus submedius. *A–D,* Caudal to rostral. Numbers in the center of each injection represent the case number (*NonSm1–13*). In NonSm7, an injection was made at 2 different anterior–posterior coordinates (*7A* and *7P*). Scale bar, 1 mm.

In contrast, within the caudal medulla, many neurons were labeled in the superficial dorsal horn of rostral nucleus caudalis following either injections of FG or Fast blue. Many labeled neurons were also seen in nucleus interpolaris. Dostrovsky et al. (1989) also reported that many neurons were retrogradely labeled in nucleus interpolaris following iontophoretic injections of WGA-HRP into Sm of rats.

In 13 rats, our injections of FG missed Sm. These injections were found in surrounding nuclei, including the rhomboid nucleus, nucleus reuniens, and the ventromedial nucleus. In each case, small numbers of neurons were labeled in the spinal cord. Again, the majority of labeled neurons were located in the deep dorsal horn and intermediate zone/ventral horn and very few neurons were labeled in the marginal zone. These results are consistent with previous studies in rats in which injections of HRP or fluorescent retrograde tracers into the medial thalamus labeled neurons that were located almost exclusively in deep areas of the gray matter (Giesler et al., 1979, 1981; Kevetter and Willis, 1983; Menetrey et al., 1984; Coffield and Miletic, 1987; Nahin, 1988). Thus, based on these studies using a variety

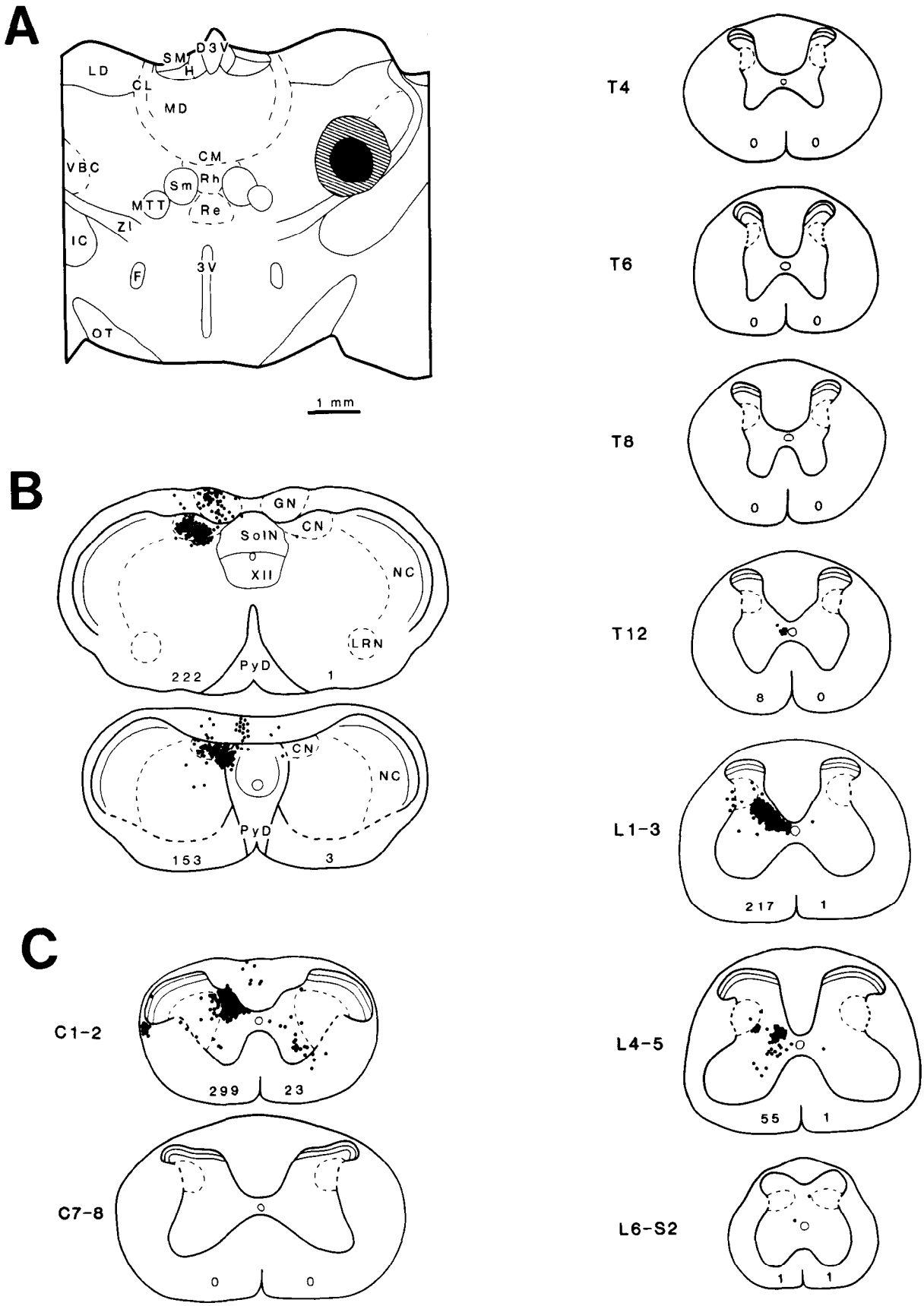

Figure 5. Reconstruction of an injection of FG into the ventrobasal complex and the locations and numbers of neurons retrogradely labeled in alternate sections of the caudal medulla and spinal cord. *A*, Injection site. Scale bar, 1 mm. *B*, Locations and numbers of labeled neurons in the caudal medulla. *C*, Locations and numbers of neurons labeled in 16 spinal cord segments. C4-5 is not included but contained one neuron in SDH.

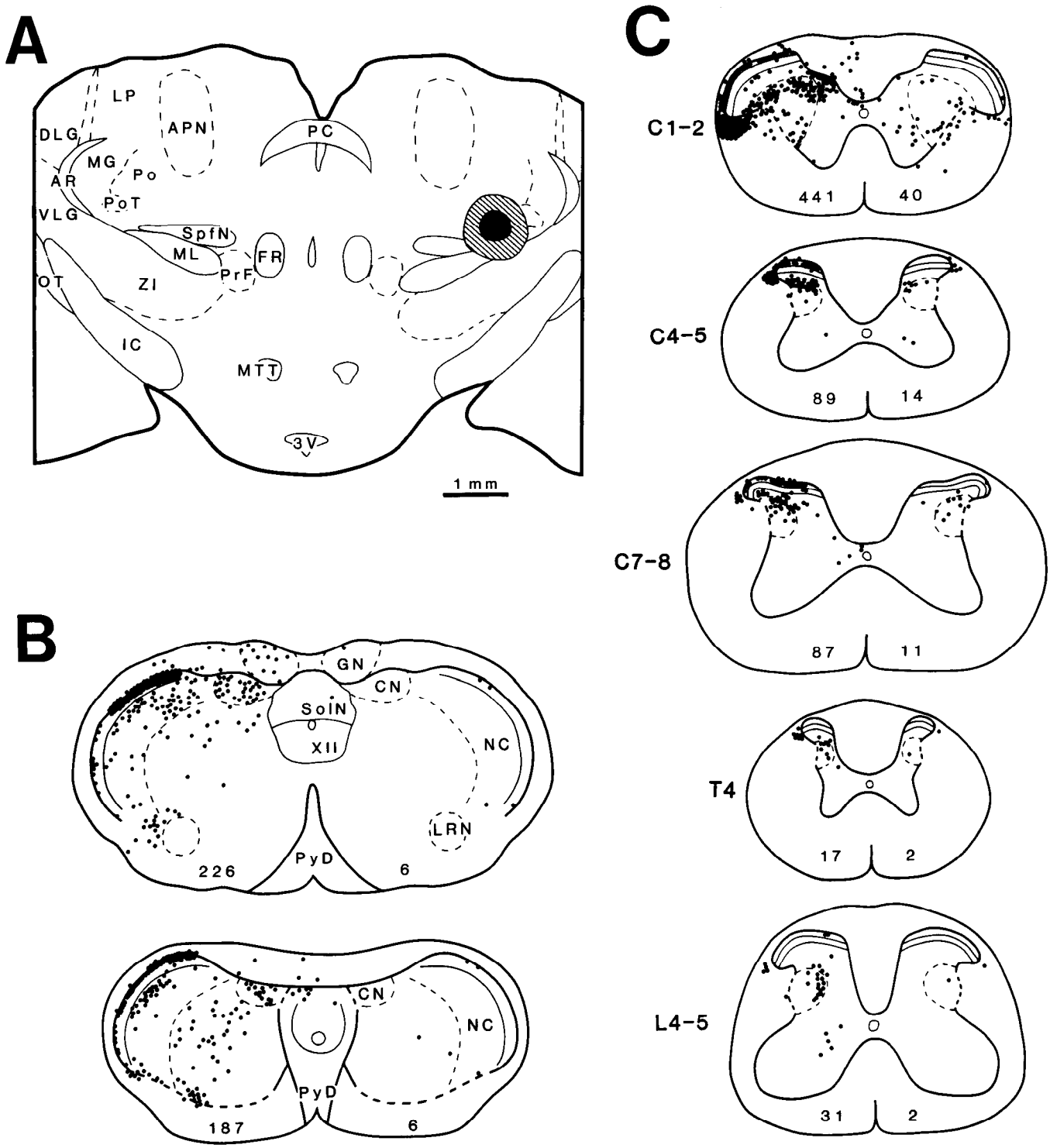

Figure 6. Reconstruction of an injection of FG into the area of the posterior thalamic group and the locations and numbers of neurons retrogradely labeled in alternate sections of the caudal medulla and spinal cord. *A,* Injection site. Scale bar, 1 mm. *B,* Locations and numbers of labeled neurons in the caudal medulla. *C,* Locations and numbers of labeled neurons in 9 representative spinal cord segments. T6, T8, T12, L1-3, and L6-S2 are not included but contained 1, 1, 5, 13, and 1 labeled neuron(s), respectively.

of tracers, it appears that there is a small, diffuse projection from neurons located in deeper areas of the spinal cord gray matter to Sm and the nuclei near it. Almost no neurons in the marginal zone of the spinal cord appear to project to this region of the thalamus in rats.

A recent electrophysiological study in rats (Dostrovsky and Guilbaud, 1990) has shown that there are nociceptive neurons throughout the medial thalamus, including the medial dorsal nucleus, anterior medial nucleus, ventral medial nucleus, ventral lateral nucleus, and the intralaminar nuclei. Within these nuclei, the percentage of nociceptive neurons is similar to that found in Sm. In addition, many neurons in these nuclei have

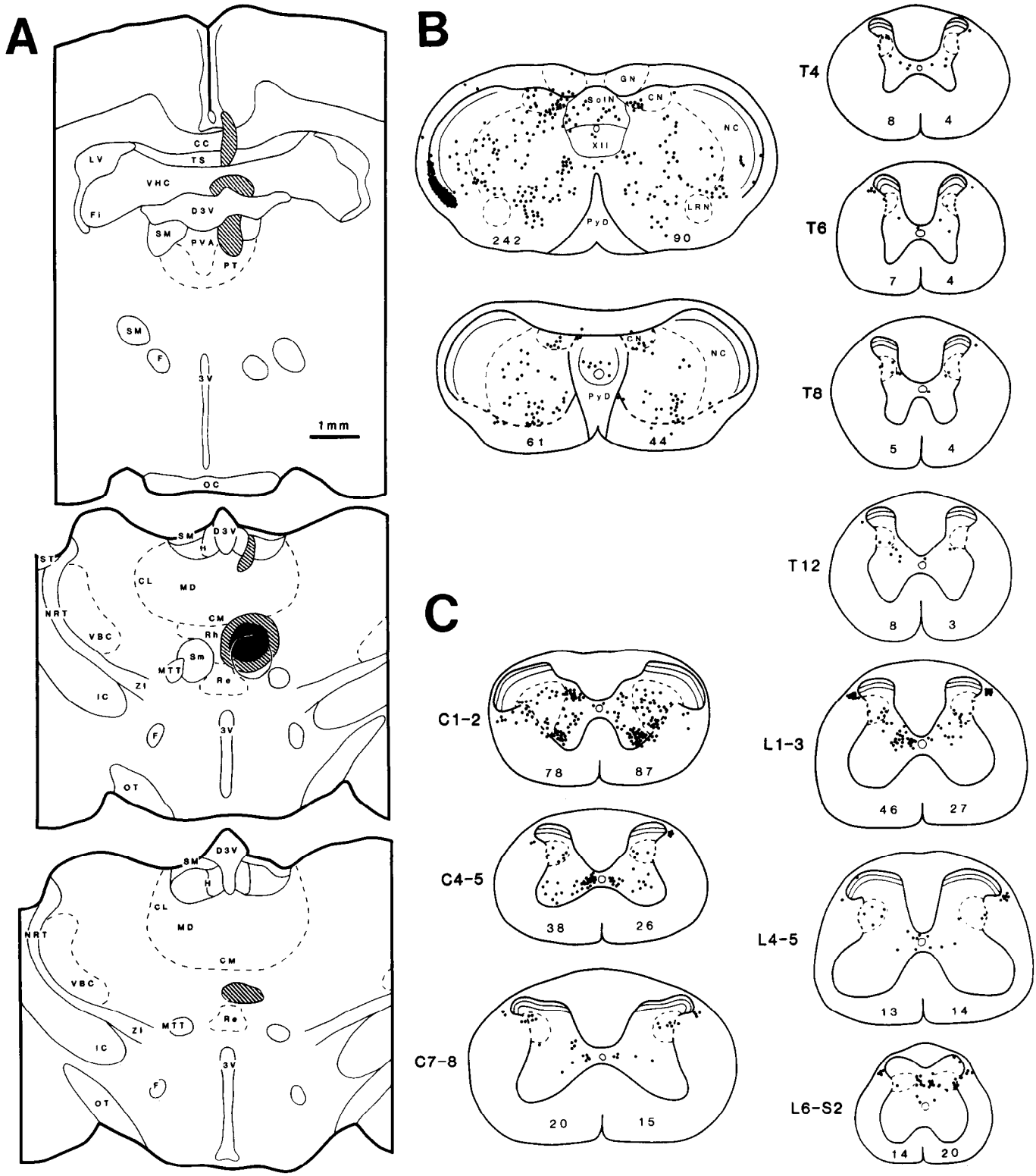

Figure 7. Reconstruction of a pressure injection (*Sm8*) of Fast blue into nucleus submedius and locations and numbers of retrogradely labeled neurons in alternate sections of the caudal medulla and 18 spinal cord segments. *A,* Injection site. Anterior limit of the injection is shown at *top* and posterior limit at the *bottom.* Scale bar, 1 mm. *B,* Locations and numbers of labeled neurons in the caudal medulla. *C,* Locations and numbers of labeled neurons in the spinal cord.

response characteristics and receptive fields that are virtually identical to those of neurons in Sm (Dostrovsky and Guilbaud, 1988, 1990). Therefore, neurons within Sm and several other medial thalamic nuclei have similar response characteristics and receive similar projections from the spinal cord.

Technical considerations

Our iontophoretic injections of FG into Sm consistently labeled only a small number (<100) of neurons in the spinal cord and almost none in the marginal zone. These results might suggest

that our tracing techniques were inadequate for labeling all neurons that project to Sm. For the following reasons, we believe the techniques we used were appropriate and effective: (1) Very similar results were obtained following injections of 2 different fluorescent retrograde tracers, FG and Fast blue. These tracers appear to be among the most sensitive for labeling projection neurons in the spinal cord (Schmued and Fallon, 1986; Burstein et al., 1987, 1990a, b; Craig et al., 1989a, b). It has also been reported that Fast blue labels small neurons within the marginal zone more effectively than does HRP (Craig et al., 1989a). (2) Injections of FG into the ventrobasal complex that were similar in size to those into Sm labeled hundreds of neurons in the spinal cord. (3) Small iontophoretic injections of FG near the border between VBC and Po also labeled many neurons in the spinal cord, including more than 100 in the marginal zone in the most effective of the 3 cases.

The spinal and trigeminal inputs to nucleus submedius are somatotopically organized in cats and, to a lesser degree, in rats (Craig and Burton, 1981). Therefore, it is possible that we failed to label many neurons in the spinal cord because our injections missed the area of Sm that receives spinal input (i.e., the rostral region of the nucleus). We believe this is unlikely since each injection covered more than three-quarters of the rostrocaudal extent of Sm. In all cases, the injection spread anteriorly to the rostral limit of Sm. Also, the injections into Sm were centered at different anterior–posterior levels of the nucleus. No clear differences could be seen regarding the quantity or distribution of neurons labeled in these cases.

Craig et al. (1989b) have suggested that to achieve maximum labeling of lumbosacral neurons following injections of Fast blue in cats, survival periods of 1 d/cm of transport are needed. Therefore, we allowed 2 rats (Sm8, 9) to survive 11 d following injections of Fast blue into Sm to determine if a larger number of neurons would be labeled in the marginal zone following this long survival time. This survival period should have allowed transport of the tracer to the lumbar cord, since the distance from the thalamus to the lumbar enlargement in rats is approximately 10–11 cm. Following these fairly large injections, a greater total number of neurons was labeled throughout the length of the spinal cord, but there was no increase in the percentage of neurons labeled in the superficial dorsal horn. Fewer than 2% of the labeled neurons were in the marginal zone. Thus, in our experiments, the distribution of the locations of neurons projecting to Sm from the spinal cord was not altered by longer survival times.

The findings of Craig et al. (1989b) raise the possibility that other fluorescent tracers, including FG, might also require longer survival periods for adequate transport throughout the spinal cord. Following injections of FG into Sm, rats were allowed to survive 3–5 d. The distance from the thalamus to the cervical enlargement in rats is approximately 4–5 cm. Therefore, these survival times of 3–5 d should have been more than adequate for the tracer to be transported through at least the cervical cord. If these survival periods were only sufficient to label marginal zone neurons in the cervical cord, it would be predicted that these neurons would be found predominantly in this region of the cord. Yet, only 2 of the 4 labeled neurons counted in the marginal zone of the spinal cord were located in the cervical segments. The other 2 were in the lumbar enlargement. Therefore, the distribution of labeled neurons in the marginal zone does not suggest that the survival times we used were insufficient for transport of the tracer to the lumbar cord. Finally, in previous studies using survival periods similar to those in the present study, FG labeled thousands of spinohypothalamic tract (Burstein et al., 1990a) and spinothalamic tract (Burstein et al., 1990b) neurons throughout the length of the spinal cord, including hundreds within the marginal zone. Thus, the survival times used in this study appear to have been adequate.

Other species

In several respects, the input to Sm from the spinal cord and caudal medulla is similar in rats and cats. The numbers of neurons in the spinal cord projecting to Sm appear to be small in both species. In the 2 cases in the cat presented by Craig and Burton (1981), totals of 77 and 300 neurons were counted in the 15 spinal cord segments examined. The rostrocaudal distribution of labeled neurons in the spinal cord also appears to be similar in both species. The majority of labeled neurons are located in the upper cervical cord. In the present study, nearly 75% were in the cervical cord, and Craig and Burton (1981) also reported that their most restricted injections into Sm labeled neurons predominantly in the upper cervical cord. In both species, the number of neurons that project to Sm from the spinal trigeminal nuclei is greater than the number that project from the entire spinal cord. Also, in both rats and cats, the majority of neurons in nucleus caudalis that project to Sm are located in the marginal zone.

In contrast, the locations of neurons in the spinal cord projecting to Sm in rats and cats differ in several respects. In cats, the input to Sm originates predominantly from neurons in the marginal zone (Craig and Burton, 1981). However, the input to Sm from the spinal cord in rats arises almost exclusively from neurons deep in the dorsal horn or the intermediate zone/ventral horn. Menetrey et al. (1984) illustrated an injection of HRP into Sm and the neurons that it labeled in a single rat. The few labeled neurons were located deep in the lumbar dorsal horn. Craig and Burton (1981) also reported that there is a minimal ipsilateral projection to Sm from neurons in the spinal cord of cats. In the present study in rats, approximately 40% of the neurons labeled by injections into Sm were in the ipsilateral spinal cord. Peschanski et al. (1983) reported that injections of WGA-HRP into the spinal cord of rats anterogradely labels spinothalamic tract axons bilaterally in Sm. This bilateral input from the spinal cord may contribute to the production of the large, bilateral receptive fields that characterize many of the neurons in Sm of rats (Dostrovsky and Guilbaud, 1988; Miletic and Coffield, 1989).

Recent anatomical studies led Apkarian and Hodge (1989a, b) to suggest that the input to Sm from the spinal cord may also be small in monkeys. Following injections of WGA-HRP into the lumbar cord, the authors found a small amount of what they interpreted to be terminal labeling within Sm. In contrast, labeled axons in the central lateral and dorsal medial nuclei were found to be considerably more numerous and covered a much larger area. Also, Mantyh (1983a) reported that the spinal cord projection to Sm in monkeys was "light to moderate." However, others have reported denser terminal labeling in Sm of monkeys following injections of HRP into the spinal cord (Craig and Burton, 1981; Burton and Craig, 1983). Apkarian and Hodge (1989a) also have reported that large injections of WGA-HRP into the thalamus, that included Sm, resulted in fewer labeled neurons in the marginal zone of the spinal cord than did injections which excluded Sm. It would be valuable to examine directly the spinal projections to Sm in monkeys using restricted injections of retrograde tracers.

Table 1. Mean (±SD) numbers of neurons retrogradely labeled in alternate sections in nucleus caudalis and the spinal cord following iontophoretic injections of FG into nucleus submedius of 6 rats

	NC	C1-2	C4-5	C7-8	T4
SDH	63.2 ± 65.9	0.3 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
DDH	15.0 ± 14.9	30.7 ± 28.9	3.0 ± 5.5	1.3 ± 2.4	1.0 ± 0.6
IZ/VH		17.3 ± 13.2	2.8 ± 4.7	1.2 ± 1.9	1.0 ± 1.3
ACC		2.2 ± 1.2	2.3 ± 2.3	2.2 ± 3.1	0.7 ± 1.0
LSN/LCN		1.7 ± 1.9	0.7 ± 1.0	1.3 ± 1.5	0.2 ± 0.4
Total	78.2 ± 76.6	52.2 ± 43.2	8.8 ± 12.7	6.0 ± 7.3	2.8 ± 2.6

For abbreviations, see the Appendix.

Table 2. Numbers of neurons retrogradely labeled in alternate sections in nucleus caudalis and 18 spinal cord segments following injections of FG into areas near nucleus submedius of 13 rats

Case		NC	SDH	DDH	IZ/VH	ACC	LSN/LCN	Total in spinal cord
NonSm	1	7	7	61	50	23	11	152
	2	12	4	37	46	18	13	118
	3	16	1	25	19	6	10	61
	4	14	17	40	30	18	31	136
	5	10	4	12	10	10	12	48
	6	4	2	13	16	3	2	36
	7	94	1	86	65	20	27	199
	8	0	0	28	9	0	0	37
	9	54	2	16	18	10	4	50
	10	37	0	16	10	8	1	35
	11	6	1	68	16	8	63	156
	12	11	0	27	38	20	5	90
	13	24	0	16	11	10	1	38

For abbreviations, see the Appendix.

Functional considerations

Only a single electrophysiological report has appeared in which spinal or medullary inputs to Sm have been carefully identified and characterized. Dostrovsky et al. (1987) examined the response properties of neurons in the superficial area of nucleus caudalis that project to Sm in cats. This study is of particular interest since the majority of input to Sm in rats (present study) and cats (Craig and Burton, 1981) originates in the spinal trigeminal nuclei. Approximately 14% of the neurons recorded were classified as nociceptive-specific. In contrast, 81% of the recorded neurons responded only to innocuous cooling of their receptive fields (Dostrovsky et al., 1987). These findings suggest that the large, direct afferent projection from nucleus caudalis to Sm primarily carries information regarding innocuous changes in the temperature of the skin.

Recently, we determined the total number of spinothalamic tract neurons in rats (Burstein et al., 1990b). Injections of FG were made that virtually filled the thalamus unilaterally. Following a 4–5 d survival period, the same 18 spinal cord segments

Table 3. Numbers of neurons retrogradely labeled in alternate sections in nucleus caudalis and the spinal cord following pressure injections of Fast blue into nucleus submedius of 3 rats

	NC	C1-2	C4-5	C7-8	T4	T6	T8	T12	L1-3	L4-5	L6-S2	Total in spinal cord
Sm7												
SDH	69	1	0	0	0	0	0	0	0	0	0	1
DDH	6	8	2	3	0	1	0	2	0	0	1	17
IZ/VH		17	7	1	1	0	0	0	1	0	0	27
ACC		3	3	5	0	0	0	0	1	1	1	14
LSN/LCN		3	1	0	0	0	0	3	3	0	0	10
Total	75	32	13	9	1	1	0	5	5	1	2	69
Sm8												
SDH	89	0	0	0	0	0	0	0	0	1	2	3
DDH	21	94	11	17	5	4	7	8	28	9	22	205
IZ/VH		63	32	5	3	1	0	1	17	3	0	125
ACC		3	14	7	3	1	1	1	10	6	3	49
LSN/LCN		5	7	6	1	5	1	1	18	8	7	59
Total	110	165	64	35	12	11	9	11	73	27	34	441
Sm9												
SDH	56	1	0	3	1	0	0	0	0	2	1	8
DDH	4	36	15	8	5	2	3	9	12	17	12	119
IZ/VH		44	25	3	2	2	1	2	7	2	2	90
ACC		4	18	6	0	1	1	4	5	5	1	45
LSN/LCN		6	9	2	2	0	0	0	6	5	3	33
Total	60	91	67	22	10	5	5	15	30	31	19	295

For abbreviations, see the Appendix.

Table 1. Continued

T6	T8	T12	L1-3	L4-5	L6-S2	Total in spinal cord
0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.8	0.0 ± 0.0	0.7 ± 0.8
1.2 ± 1.0	1.0 ± 1.3	1.5 ± 1.2	4.5 ± 5.1	2.0 ± 3.5	0.3 ± 0.5	46.5 ± 44.8
0.3 ± 0.5	0.3 ± 0.5	1.0 ± 1.1	2.8 ± 1.9	0.2 ± 0.4	0.0 ± 0.0	27.0 ± 20.1
0.2 ± 0.4	0.0 ± 0.0	0.5 ± 0.8	2.0 ± 2.9	0.2 ± 0.4	0.0 ± 0.0	10.2 ± 9.30
0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.7 ± 1.2	0.8 ± 1.6	0.5 ± 0.8	6.0 ± 7.2
1.7 ± 1.4	1.5 ± 1.4	3.0 ± 2.5	10.0 ± 7.4	3.5 ± 5.3	0.8 ± 1.0	90.3 ± 79.0

examined in the present study were removed, and labeled neurons were counted in alternate sections. An average of more than 4000 neurons was counted in the successful cases. In the present study using the same tracer and identical counting techniques, injections into nucleus submedius labeled an average of fewer than 100 neurons. A mean of approximately 420 neurons was counted in the superficial dorsal horn following injections that filled the thalamus (Burstein et al., 1990b). In contrast, the mean number of superficial dorsal horn neurons labeled following injections of FG that were restricted to Sm was less than 1. Therefore, it appears that in the rat, fewer than 5% of all spinothalamic tract neurons project to Sm. Similarly, fewer than 1% of spinothalamic tract neurons in the marginal zone project to Sm.

Finally, it should be noted that although each of our total of more than 25 injections of retrograde tracers into the thalamus labeled neurons in the spinal cord, only those at the border of the posterior VBC and Po labeled large numbers of neurons in the marginal zone. As suggested previously by Menetrey et al. (1984), it might be useful to explore the input from the spinal cord to this area in more detail.

Appendix

Abbreviations

ACC	area around the central canal
AM	anterior medial nucleus
APN	anterior pretectal nucleus
AR	acoustic radiation
C	cervical spinal cord
CC	corpus callosum
CL	central lateral nucleus
CM	central medial nucleus
CN	cuneate nucleus
DC	dorsal columns
DDH	deep dorsal horn
DH	dorsal horn
DLF	dorsal lateral funiculus
DLG	dorsal lateral geniculate nucleus
F	fornix
Fi	fimbria hippocampus
FR	fasciculus retroflexus
GN	gracile nucleus
H	habenula
IC	internal capsule
ICP	inferior cerebellar peduncle
IZ	intermediate zone
L	lumbar spinal cord
LCN	lateral cervical nucleus
LD	lateral dorsal nucleus
LF	lateral funiculus
LP	lateral posterior nucleus
LRN	lateral reticular nucleus
LSN	lateral spinal nucleus
LV	lateral ventricle
MD	medial dorsal nucleus
MG	medial geniculate nucleus
ML	medial lemniscus
MTT	mammillothalamic tract
NC	nucleus caudalis
NI	nucleus interpolaris
NRT	nucleus reticularis thalami
OC	optic chiasm
OT	optic tract
PC	posterior commissure
Po	posterior thalamic group
PoT	posterior thalamic group, triangular
PrF	prerubral field
PT	paratenial thalamic nucleus
PV	paraventricular thalamic nucleus
PVA	paraventricular thalamic nucleus, anterior
PVH	paraventricular hypothalamic nucleus
PyD	pyramidal decussation
Re	nucleus reuniens
Rh	rhomboid nucleus
SDH	superficial dorsal horn
SM	stria medullaris
Sm	nucleus submedius
SO	supraoptic nucleus
SolN	nucleus of the solitary tract
SOX	supraoptic decussation
SpfN	subparafascicular nucleus
ST	stria terminalis
StN	subthalamic nucleus
STR	superior thalamic radiation
STT	spinothalamic tract
STV	spinal trigeminal tract
T	thoracic spinal cord
TS	triangular septal nucleus
VBC	ventrobasal complex
VH	ventral horn
VHC	ventral hippocampal commissure
VLG	ventrolateral geniculate nucleus
ZI	zona incerta
3V	third ventricle
D3V	dorsal third ventricle
XII	hypoglossal nucleus

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