นิพนธ์ต้นฉบับ

Anatomical localization of interneurons projecting to spinocerebellar tract cells in guinea pigs : a study of retrograde transneuronal transport of WGA-HRP.

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The localization of interneurons of the spinocerebellar system in the lumbar and sacral spinal cord of five guinea pigs was studied by injection of WGA-HRP (wheat germ agglutinin conjugated with horseradish peroxidase) into the cerebellar vermis. The interneurons were found in lamina III of L_4 - L_5, laminae IV-VI of L_1-S, the central part of lamina VII of L_1-S, lamina VIII of L_1-S and lamina IX of L_2-L_5. The interneurons were 20-30 μm in size, and round, oval, triangular and spindle in shape. the locations of the interneurons were nearly the same as for spinocerebellar tract neurons, except for laminae III-VI of L_5-L_6, the lateral part of lamina VII of L_6 and laminae IV-V of sacral segments, in which were found only interneurons. In lamina VIII, even though there were spinocerebellar tract neurons, the amount was less than that of interneurons. Interneurons were frequently found in laminae IV-VI, VII, VIII which were the same as interneurons projecting to the hind-limb motoneurons. From this study and previous electrophysiological evidence, it is concluded that the interneurons projecting to spinocerebellar tract neurons are the same group as those projecting to the hind-limb motoneurons.

Key words : *WGA-HRP, Interneurons spinocerebellar tract cell.*

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วิไล ชินธเนศ, ธันวา ตันสถิตย์, จันทรวรรณ แสงแข. ตำแหน่งของอินเตอร์นิวรอนที่ส่งเส้นใยประสาทไปควบคุมเซลล์ประสาทสไปโนซีรีเบลล่าร์ในหนูตะเภา. จุฬาลงกรณ์เวชสาร 2536 เมษายน; 37(4) : 249-258

ได้ทำการศึกษาตำแหน่งของอินเตอร์นิวรอนของระบบสไปโนซีรีเบลล่าร์ในไขสันหลังระดับลัมบาร์ และเซครัลในหนูตะเภาโดยการฉีดสาร WGA-HRP (Wheat Germ Agglutinin conjugated with Horse Radish Peroxidase) เข้าไปในสมองส่วนซีรีเบลล่าร์ เวอร์มีส จำนวน 5 ตัว พบอินเตอร์นิวรอนใน laminal III ระดับ L_4 ถึง L_6, lamina IV-VI ระดับ L_1 ถึง S, ด้านในของ lamina VII ระดับ L_1 ถึง S, ด้านนอกของ lamina VII ระดับ L_1 ถึง S, lamina VIII ระดับ L_1 ถึง S, lamina IX ระดับ L_2 ถึง L_5 ขนาดของอินเตอร์นิวรอนอยู่ในช่วย 20-30 μm รูปร่างมีหลายแบบได้แก่ รูปกลม, รูปไข่, รูปสามเหลี่ยม และหัวท้ายกลม ตำแหน่งของอินเตอร์นิวรอนส่วนมากอยู่ในตำแหน่งเดียวกับสไปโนซีรีเบลล่าร์นิวรอน ยกเว้น lamina III-VI ในระดับ L_5 ถึง L_6, ด้านนอกของ Lamina VII ระดับ L_6 lamina IV-V ระดับ S ซึ่งพบเฉพาะอินเตอร์นิวรอนเท่านั้น และ lamina VIII แม้ว่าจะพบสไปโนซีรีเบลล่าร์นิวรอนบ้าง แต่ก็น้อยมากเมื่อเทียบกับอินเตอร์นิวรอน ตำแหน่งที่พบอินเตอร์นิวรอนมากคือ lamina IV-VI, VII, VIII ซึ่งเป็นตำแหน่งเดียวกับที่พบอินเตอร์นิวรอนที่ควบคุมมอเตอร์นิวรอนของขาหลัง จากผลการศึกษาครั้งนี้และการศึกษาทางประสาทสรีรวิทยาที่ผ่านมาแล้ว ทำให้พอสรุปได้ว่า อินเตอร์นิวรอนของระบบสไปโนซีรีเบลล่าร์และของมอเตอร์นิวรอนของขาหลังน่าจะเป็นกลุ่มเดียวกัน

The dorsal spinocerebellar tract (d.s.c.t) and its homologue, the cuneocerebellar tract (c.c.t), have been considered as forwarding information primarily on peripheral events,[1] while the main task of the ventral and rostral spinocerebellar tracts (v.s.c.t and r.s.c.t) would be to inform the cerebellum about activity in various reflex pathways to motoneurons, since their input is via the same interneurons that inhibit or excite motoneurons.[2,3] Inhibition of d.s.c.t. cells is mediated by group I excited interneurons of Rexed's laminae V-VI of the lower lumbar segment in addition to interneurons located in the same segements as Clarke's column.[4] Since previous experiments indicated that interneurons which mediate autogenetic and synergistic inhibition of motoneurons of group I origin are among the lowere lumbar laminae V-VI interneurons,[5-7] the same interneurons might well be responsible for the two actions. Injection of wheat germ agglutinin conjugated with Horseradish Peroxidase (WGA-HRP) into the peripheral nerve of the hindlimb muscle was first transported to motoneurons soma and then, transneuronally, to interneurons in laminae V-VII ipsilaterally and interneurons in lamina VIII contralaterally.[8-11] The aim of this study is to localize the interneurons projecting to spinocerebellar tract cells (s.c.t.) by injection of WGA-HRP into the cerebellar vermis of the guinea pig.

Materials and Methods

WGA-HRP (Sigma) was injected into the cerebellar vermis of five albino guinea pigs (2-4 µl of 5% solution in saline). Transported first was retrograde from the cerebellar vermis to the s.c.t. cells and then transneuronally to interneurons. Six days after the injection, the animals were sacrificed and the transcardiac area perfused with 1% paraformaldehyde, 1.5% glutaraldehyde and 4% sucrose. The brain and spinal cord were removed and cut into serial sections of 60 µm thickness by freezing microtome. The HRP marking was demonstrated by using the tetramethylbenzidins reaction[12] and observed under light microscope with polarizing filter. Many HRP reaction products could be found in the cytoplasm of the retrograde labelling neurons (s.c.t. neurons). For identification of the internurons, we used the criteria of Jankowska and Skoog[8] as follows : more than 10 reaction products being presented and distributed evenly in the cytoplasm, with the cell containing a nucleolus. the mapping and drawing of labelling neurons were done by using camera lucida. the diameter of the neurons was measured following the method of Grant and XU.[13]

Result

The localization of s.c.t. neurons and interneurons is shown in Figures 1,3,5. In GP 47 with whole cerebellar vermis injection the s.c.t. neurons were found in Clark's column (c.c) of first to third lumbar spinal cord segments (L_1-L_3), laminae IV-VI of L_1-L_4, the medial and lateral parts of lamina VII of L_1-S, and lamina IX of L_3-L_6 (Fig.1) The morphology of s.c.t. cells is shown in Figure 2. The size and shape of the s.c.t. neurons in each lamina are summarized in Table 1. The interneurons were found in lamina III of L_4-L_6, laminae IV-VI of L_1-S, the central part of lamina VII of L_1-S and lamina IX of L_2- L_5 (Figure 2.) The morphology of the interneurons in each lamina is shown in Figure 4. Table 2 summarizes the size and shape of the interneurons

In cases GP 48, GP 49, GP 50 and GP 32, only 2 µl of WGA- HRP was injected, the localization of s.c.t. neurons and interneurons was similar to that of GP 47 but fewer in number (Figure 5).

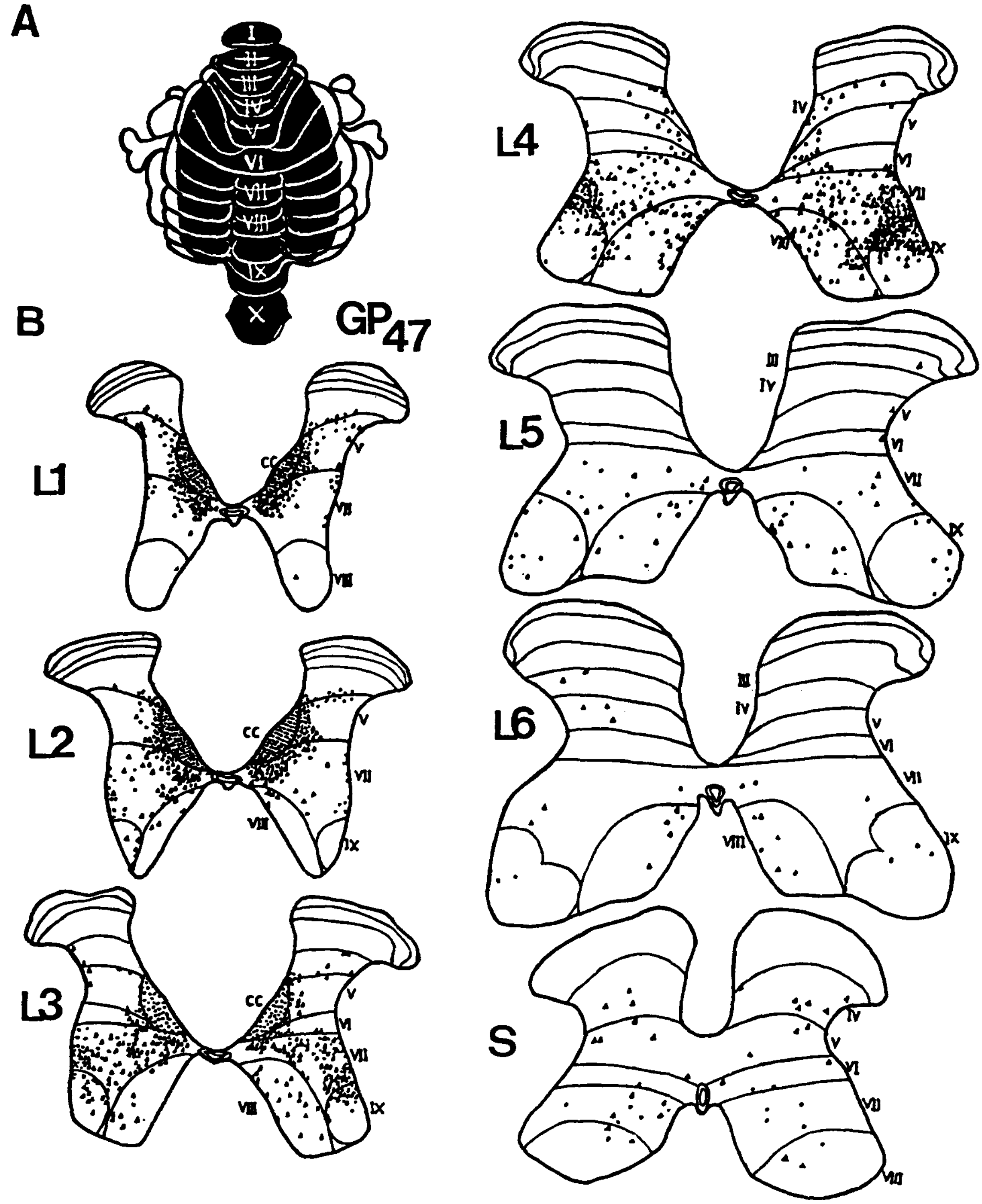

Figure 1. Diagrams showing the injections sites in the cerebellum (A) and the distribution of labeled neurons at lumbar and sacral spinal levels (B) in case GP 47. (A) unfolded cerebellar cortex, (B) Transverse sections of spinal segments L_1 to S.

. represents five SCT neurons

▲ represents one interneuron

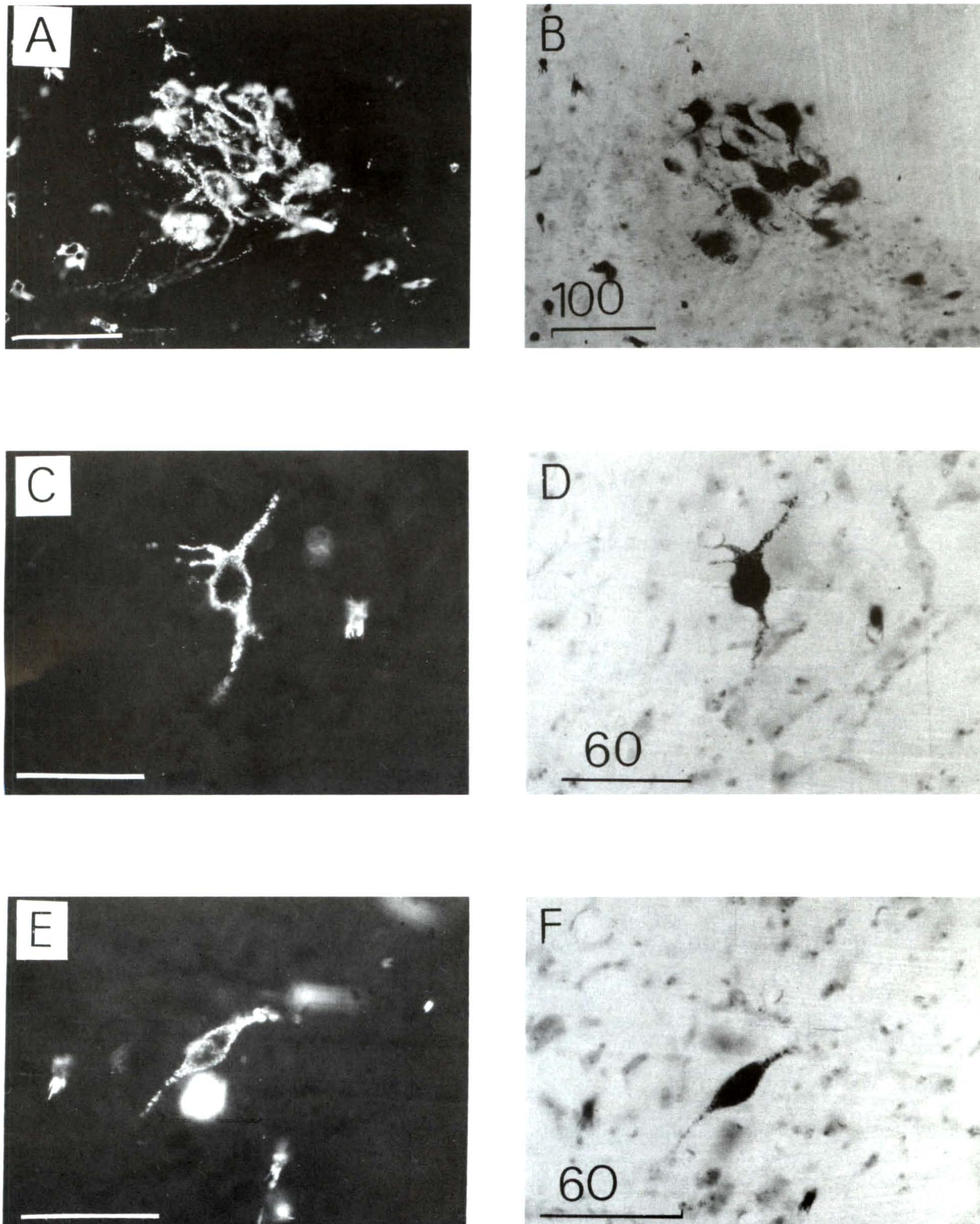

Figure 2. Photomicrograph of SCT neurons at lumbar spinal levels (L2 to L4 in case GP 47. Dark (A,C,E) and Bright illumination (B,D,F)

(A) (B) Clarke's column in L2, (C) (D) lamina IV in L2,

(D) (E) lamina V in L2

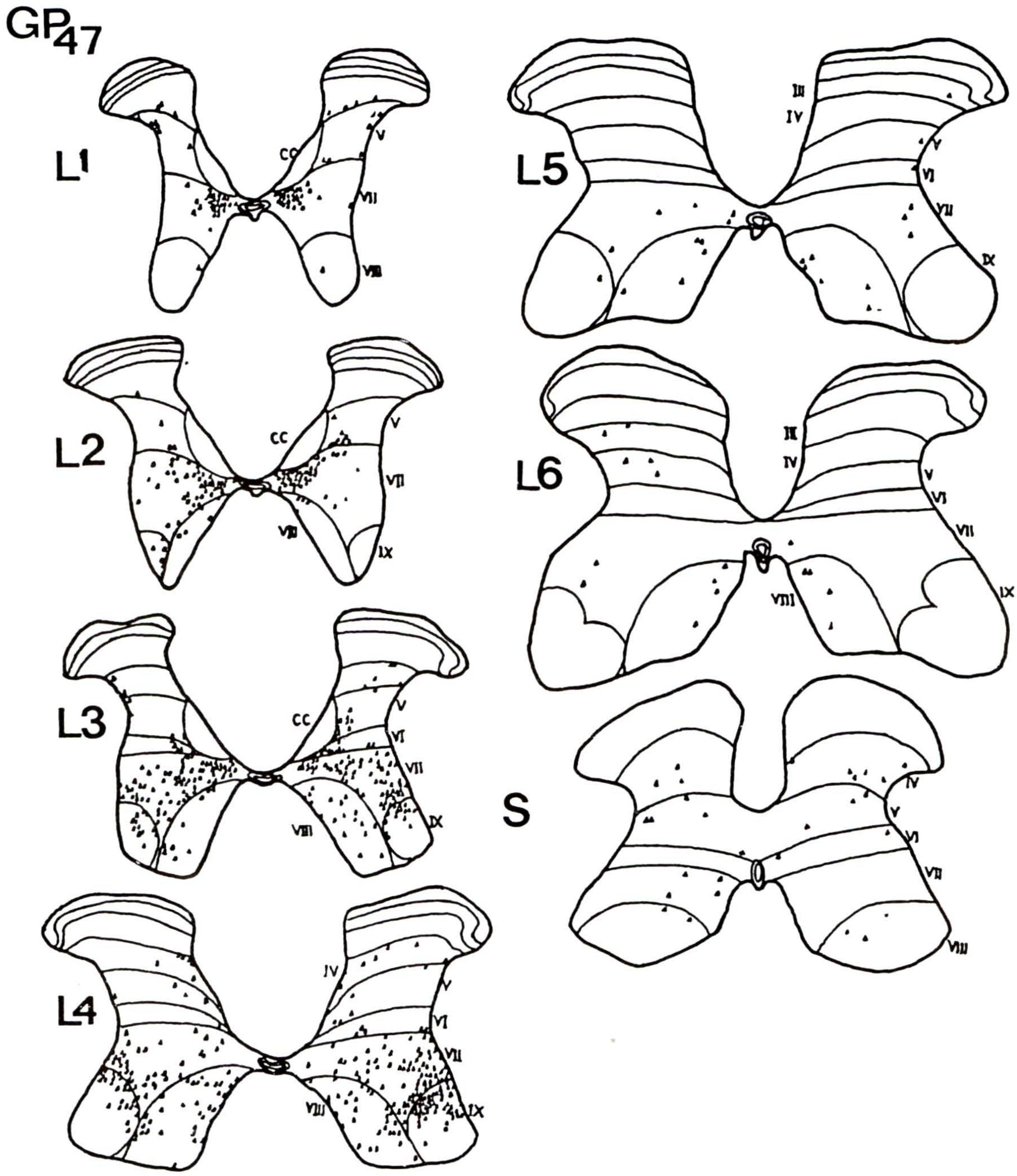

Figure 3. Diagrams showing the distribution of labeled interneurons (▲) at lumbar and sacral spinal levels in case GP 47.

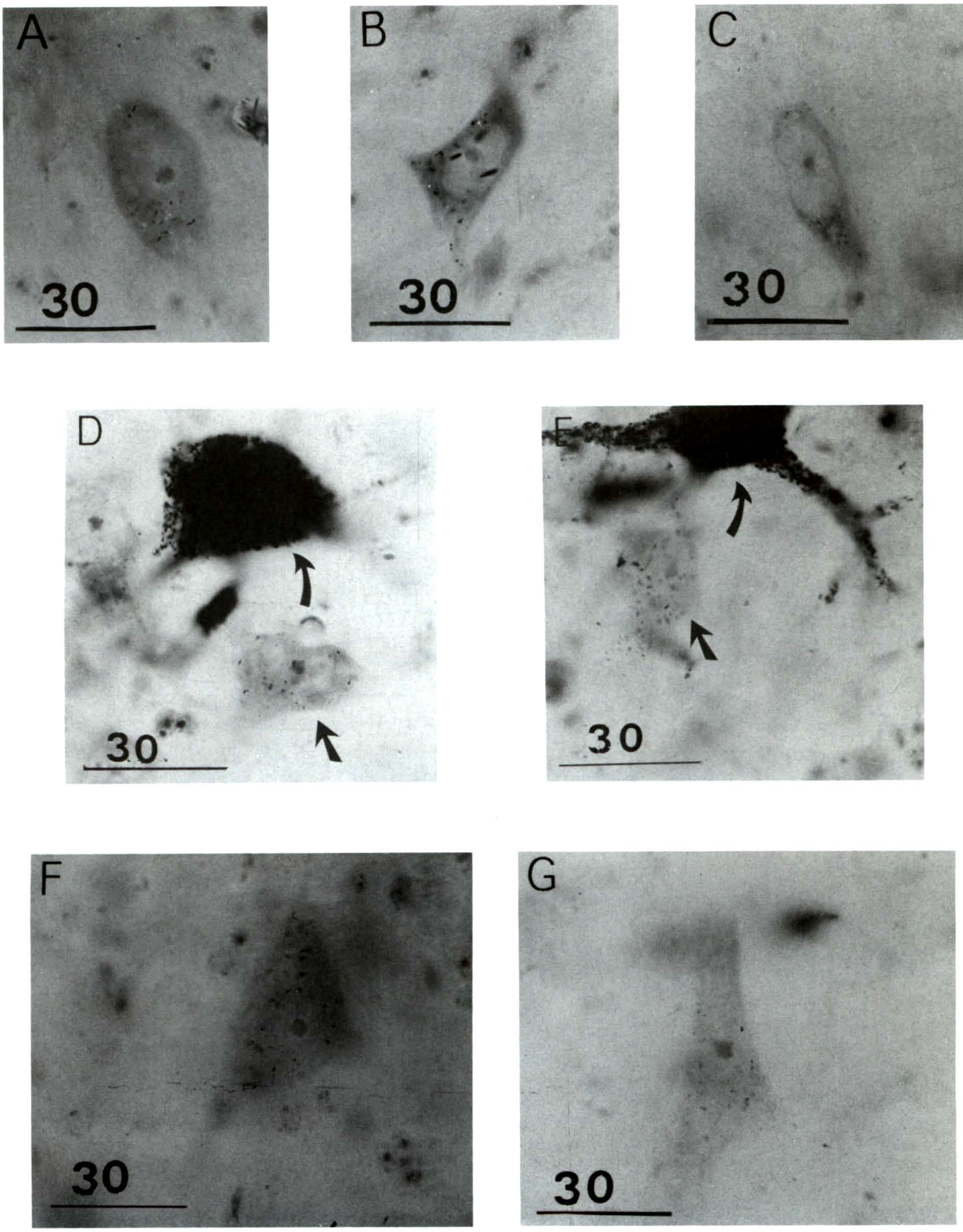

Figure 4. Photominograph of SCT neurons (↗) and interneurons (↑) in case GP47 (A) Clarke's column of L2 (B) Lamina IV of L5 (C) lamina V of L 6 (D) central of lamina VII of L3 (E) lateral of lamina VII of L3 (F) lamina VIII of L2 (G) lamina IX of L4.

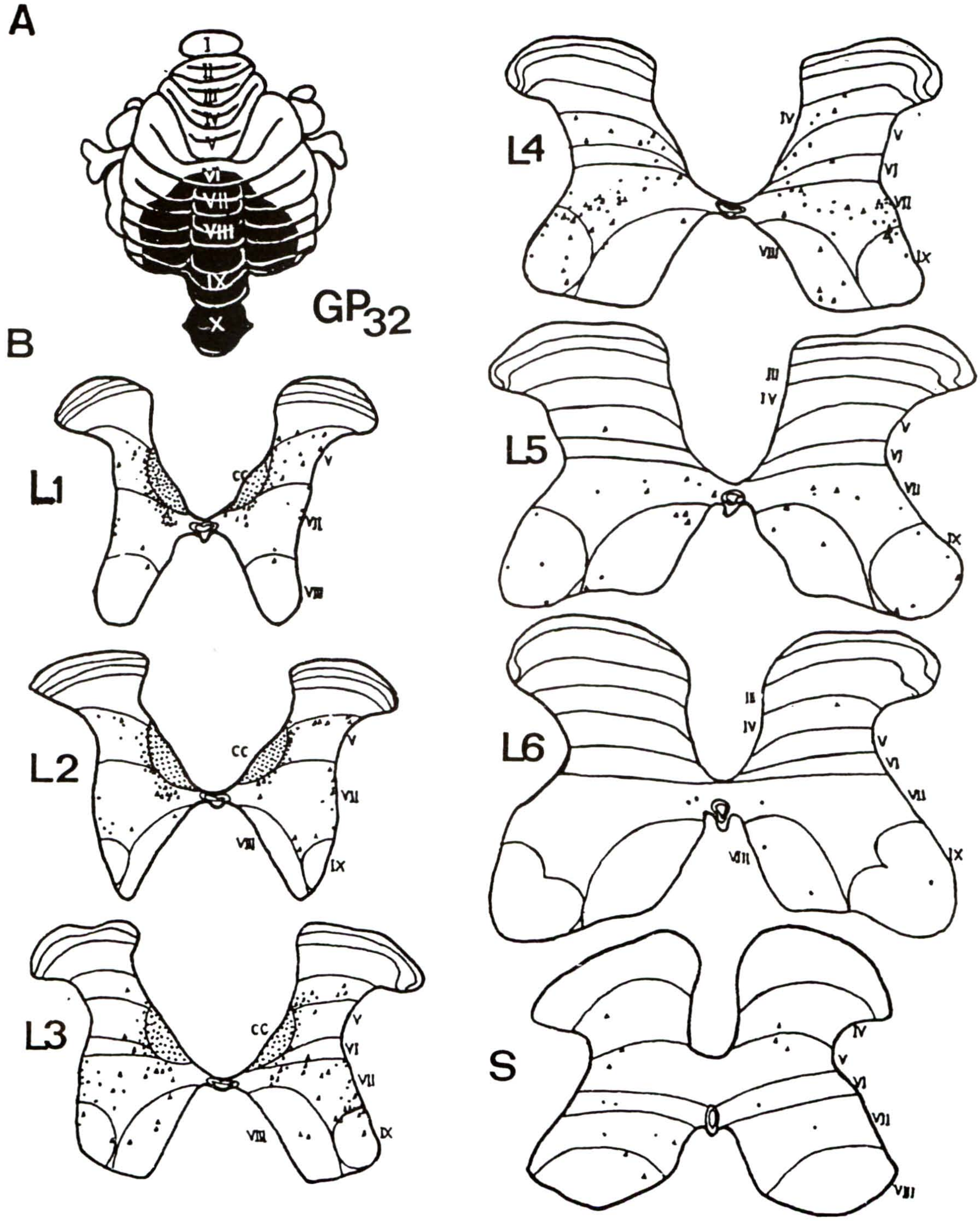

Figure 5. Diagrams showing the injections sites in the cerebellum (A) and the distribution of labeled neurons at lumbar and saeral levels (B) in case GP 32. (A) unfolded cerebellar cortex, (B) Transverse sections of spinal segments L1 to S

• represents five SCT neurons

▲ represents one interneuron

Table 1. Size and shape of s.c.t. neurones in each lamina of the lumbar and sacral spinal cord in GP 47.

Lamina	Size (μm)	Shape
Clark's column	20-35	round, oval, spindle, polygonal
IV-VI	15-35	round, oval, triangular, spindle
VII	25-58	round, oval, triangular, polygonal
VIII	28-50	round, oval, triangular, polygonal
IX	25-50	round, triangular, polygonal,

Table 2. Size and shape of interneurones in each lamina of the lumbar and sacral spinal cord in GP 47.

Lamina	Size (μm)	Shape
IV-VI	14-28	round, oval, spindle
VII	18-30	round, oval, triangular, spindle
VIII	15-30	round, oval, triangular, spindle
IX	15-28	round, oval, triangular, spindle

Discussion

The number of s.c.t. neurons and interneurons in our study depended on the amount of tracer. These neurons were frequently found in the same lamina of the spinal cord except in laminae III-VI of L_5-L_6, the lateral part of lamina VII of L_6 and laminac IV-V of sacral segments in which were found only interneurons. (Figure 1). In lamina VIII, even though there were s.c.t neurons, the amount was less than that of the interneurons. The interneurons are smaller in size than the s.c.t. neurons (tables 1,2). From this study, we could localize the interneurons projecting to s.c.t. neurons much more than those demonstrated by HRP injection to Clark's column.[4] In that report, the interneurons were found only in laminae V,VI, and VII.

The locations of interneurons projecting to s.c.t. neurons are quite similar to those projecting to motoneurons.[14] Following injection of WGA-HRP into the motor nerves, after the labelling of motoneurons, interneurons which were secondarily labelled were found in laminae V, VI, and VII ipsilaterally and lamina VIII contralaterally. Electrophysiological evidence Suggested that the same interneurons in laminae V-VI of L_6-L_7 in cats inhibited both d.s.c.t. neurons and lumbar motoneurons.[4,15-16] Interneurons in lamina VII mediated reciprocal Ia inhibition of hind-limb motoneurons,[19,20] and those in lamina VIII interposed in crossed reflex pathway.[14] From this data, it is suggested that same interneurons projected to both s.c.t. neurons and motoneurons.

Summary

The localization of interneurons projecting to s.c.t. neurons were in laminae III, IV-VI, VII, VIII and IX of the lumbar and sacral spinal cord segment. The locations were almost the same as s.c.t. neurons but the interneurons were smaller in size.

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