Terminal organization of C-fiber afferents related with nociception or non-nociception

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Abstract

The central projections of somatic, visceral and muscle unmyelinated C-fibers were examined in the guinea pig. The sensory modality of somatic C-afferent fibers were identified by natural stimuli, pinch, noxious heat and cooling. The central projections of high threshold mechanoreceptor (HTM), mechanical-cold nociceptor (MCN), polymodal nociceptor (PN) and low threshold mechanoreceptor (LTM) were observed in the cervical and lumbar cord. All somatic unmyelinated C-fibers projected into the superficial dorsal horn (laminae I, II) with a densely concentrated termination. The terminal patterns and course of fiber trajectories differed in the two levels of the spinal cord, cervical or lumbar. The visceral C-afferent fibers mainly projected to the lamina I, V, X, and contralateral V, X in the thoracic cord. Muscle C-afferent fibers from the lateral gastrocnemius were labeled in the lumbar cord. Terminal branches were located in laminae I, II and III in the several lumbar segments. The number and size of terminal swellings were compared among these C-afferent fibers to consider the nature of sensory processing.

Key words: unmyelinated fiber, central projection, somatic afferent, visceral afferent, muscle afferent

侵害受容，非侵害受容を伝達する無髄神経（C線維）の脊髓終末機構

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脊髓一次求心性神経の中で無髄神経（C線維）は主に痛覚や温度感覚に関係している。この無髄C線維は、髄鞘がないことと線維が細いことから標識することが難しく、脊髓での神経終末は有髄神経のように詳細な研究はされていない。末梢神経はHRPあるいはWGA-HRPを各種器官に投与することによ

り、中枢神経系での神経終末の形態や分布が明らかになっている^{1,2,4,5,14–16,20,24,46,66,68,94,97}。HRPを局所投与する方法に加えて、細胞内に注入することにより、単一の神経の機能と終末分枝の形態学的解析が可能になってきた。この細胞内注入法で有髄神経を持つ神経細胞の形態学的特徴と単一神経細胞の機

Table 1 Terminal domains and characteristics of each type of C-fibers

Sensory modality of receptive field	Segmental level	Cell size (μm)	Conduction velocity	Receptive fields	fiber course	Rostrocaudal extension (μm)	Dorsoventral extension (μm)	Mediolateral extension (μm)	Laminae
High threshold mechano-receptor	C2	38X16	0.5	Centrum of Pinna	Lissauer's tract	280	100	150	Ilo, part of Ili
Polymodal nociceptor	C2	30X25 42X23	0.5	Anterior of Pinna	Lissauer's tract	300	50	200	Ili
	L6		0.5	III digit of Lower leg	Dorsal funiculus	150	150	200	I,Ilo, III,IV
Mechanical cold nociceptor	C2	30X25 -	0.5	Anterior of pinna	Lissauer's tract	400	300	150	I, part of Ilo
	L6		0.5	Backside of thigh	Lissauer's tract	450	100	150	I, part of Ilo
low threshold mechano-receptor	C2	-	1.5	lateral of pinna	Lissauer's tract	380	50	120	Ilo, part of Ili
	C2	30X20	0.6	Anterior of pinna	Lissauer's tract	300	50	100	Ili-Ilo

能を同時に得ることができた^{2,10,11-13,23,28,30,35-37,47,82-84,86-88,95,96}。さらにこの方法に免疫染色を加え二重染色することにより、その終末の物質レベルまでわかることになった^{47,52,73}。

脊髄後角の中でも表層、特にI層 (marginal layer), II層 (substantia gelatinosa)⁷⁴) はGolgi法、変性法などを用い数多くの研究がなされ、細い有髄神経、無髄神経など温痛覚を伝える一次求心性神経の終末部位であることが明らかにされた^{7,17,19,44,58,69,70-72,76,96}。生理学的実験は無髄一次求心性神経線維を持つ侵害受容器、機械的受容器がII層の細胞を直接興奮させることを明らかにした^{41,46}。しかし形態学的な方向からの多くのアプローチにも関わらず、侵害刺激を伝える線維は二次ニューロンの細胞体に直接シナプス接触する事実は示されなかった。無髄求心性神経の脊髄での終末分布^{90,93})の解析の結果からも二次細胞の細胞体に一次求心性神経の終末が直接シナプスを形成することはなく、一次求心性神経終末の形成する中心終末に二次ニューロンの樹状突起が接触することによって神経伝達が行われていることが明らかにされてきた^{3,6,25,31,53,67,75,93}。また一次神経終末が放出するサブスタンスPに対するSPレセプターの脊髄後角での分布も、直接的コンタクトを示唆する同一部位での局在は見られていない³⁴。そこで、この稿では痛みに関連する一次神経終末とりわけ各種の無髄神経の終末の脊髄内分布の特徴を述べ、今後の痛覚の研究の基礎となるよう各種受容器の脊髄内神経終末の形態を記載しておく。

皮膚からの無髄一次求心性神経終末の脊髄内分布

ここで記載した無髄神経終末はいずれも雌モルモットを用いた実験結果である。ネンプタールによる深麻酔下で後根神経節細胞から細胞内記録を行い、末梢の電気刺激により伝導速度を測定し、2m/秒以下の伝導速度を示すものを無髄神経であると同定した。神経細胞のトレーサーとしてPHA-Lを細胞内に注入しPHA-Lに対する抗体で免疫染色した²⁹。末梢侵害受容器の感覚特性の同定はピンチによる機械的刺激、冷却による寒冷刺激、輻射熱による熱刺激などの自然刺激により誘導された細胞内活動電位の記録によって確認した^{8,9,40,45,48,85} (Table 1)。

1. 高閾値機械的受容器 (HTM)

頸髄2番 (C2) 後根神経節で記録した、強い機械的刺激にのみ応ずる高閾値機械的受容器 (HTM) は大後頭神経を経て耳介中央部に受容野を持っている。中枢枝はC2の高さで脊髄後根の内側より後角表面に到達した後角I, II層を通過し、III, IV層を通りながら2~3本に分岐する。その枝はいずれも後方に1分節ほど走行し、頸髄後角外側のI, II層の境界部で密な終末神経叢を作る。その終末神経叢はおおよそ吻尾方向に280μm、背腹方向に100μmであった (Fig. 1)。HTMの脊髄内分枝は走行途中、目立った終末膨大を示さず、終末神経叢を作るところでは大きさが数ミクロンにも及ぶ多数の終末膨大を形成する。

Fig. 1 A central trajectory of a high threshold mechanoreceptor of the C2 spinal cord. Receptive field was located in the center of the pinna. Conduction velocity was approximately 0.5 m/sec. (A) Sagittal view of the reconstructed termination pattern in the serial sections by camera lucida. After enter the spinal cord, the fiber runs deeper layer and the terminates in the superficial layer (lamina II). A solid line indicates the outline of the dorsal surface of the spinal cord. An arrow head indicate the origin of the fiber from the rootlet. Scale; 100 μ m. (B, C) Low magnification photomontage combined with different focused photographs and traced drawing, which shows terminal swellings in lamina II. Scale; 50 μ m. (D) A transverse view of the fiber terminals. The fiber terminated in the lateral surface of the superficial dorsal horn.

2. ポリモーダル侵害受容器 (PN)

C2 後根神経節で記録した PN は耳介前方皮膚に強い機械的刺激と熱刺激に反応する受容野を持っていた。後根神経枝は脊髄後角に到達し Lissauer 路を後方に約 2000 μ m 走行し、後角 II 層内層に密な終末分枝を形成する。II 層に形成される神経叢は吻尾方向に約 300 μ m で内外側に 200 μ m の領域に広がっている。終末膨大の大きさは 8 μ m に及ぶ (Fig. 2)。

腰髄 L6 後根神経節で記録した PN は後肢末梢の第 3 足趾に受容野を持っていた。終末分枝の走行は頸髄とは全く異なり、脊髄表面に達すると吻尾方向に分枝し、追跡できた長さは尾方の分枝が約 1 mm、吻側の分枝は約 3.5 mm であった。この神経は走行途中 200 ~ 400 μ m 間隔で側枝を十数本出していた。主な神経叢は数本の側枝から構成され、後根神経の入った高さで吻尾方向に約 600 μ m、内外側に 200 μ m

の広がりを持ち、I 層と II 層の外層に集中していた。側枝のほとんどは短く、密な終末膨大をもたない。側枝の中の 1 本のみが終末を III, IV 層に出していた。終末膨大の大きさは最大長軸で 7.5 μ m に達していた (Fig. 3)。

3. 機械的寒冷侵害受容器 (MCN)

C2 頸髄から記録、標識された MCN は耳介前部の皮膚に機械刺激と寒冷刺激に応じる受容野を持っていた。中枢枝は脊髄に入り、ほぼ 1 分節後方に走行し、前、後に側枝を出す。前後の側枝はともに脊髄後角、外側の I, II 層の境界領域で密な神経叢を作り、終末領域を形成した。この終末領域は吻尾方向に約 400 μ m、内外側に 150 μ m に展開していた。終末膨大の大きさは約 3.5 μ m 以下であった (Fig. 4)。一方、腰髄の MCN は脊髄に進入したレベルで密な神経叢を形成し、吻尾方向で約 450 μ m、内外側で

Fig. 2 The terminal distribution of a polymodal nociceptor in the C2 spinal cord. Receptive field was located in the anterior skin of the left pinna. (A) The sagittal view of the fiber was reconstructed by serial sections. Upon entering the spinal cord, the fiber runs caudally in Lissauer's tract about 2000 μm and terminates in inner lamina II. Scale; 100 μm . (B, C) Photomontage of the fiber terminals and drawings traced from the same terminal field in III. The termination was constituted of concentrated terminal swellings which shows en passant or end terminals. Scale; 50 μm . (D) Transverse view of the fiber termination. Terminal plexus is located in inner lamina II after penetrating the lateral margin of the cervical spinal cord.

Fig. 3 Reconstructed views of a C polymodal nociceptor in the L6 spinal segment. Receptive field was located on the lateral skin of the third toe. (A) Sagittal view of the computer reconstructed termination pattern. Upon entering the spinal cord, the fiber bifurcated into a descending and an ascending branch. The descending branch was shorter (approximately 1080 μm) than the ascending (truncated by the rostral limit of the tissue block at 3550 μm). Most of the terminal arborization and boutons were located in laminae I and II. A few collaterals with small arborizations were found in laminae III and IV. Scale 200 μm . (B) Low magnification photomontage showing the labeled fiber and the terminal swellings in laminae I and II. Scale 50 μm . (C) Transverse view rotated by computer reconstruction. Most of the terminal arborizations were located in a narrow mediolateral sector of laminae I and II. Scale 200 μm . (D) Transverse drawing showing the terminal arborization of the fiber in relation to various laminae of the dorsal horn.

Fig. 4 Computer reconstruction of the central branches of a C-mechanical-cold nociceptor of the C2 segment using the three-dimensional interactive graphic program. The sagittal view of the fiber was reconstructed from 20 sagittal 50 μ m sections. The fiber ran caudally in Lissauer's tract to the C3 level, where several branches merged into a terminal plexus. The termination area extended to 400 μ m rostrocaudally and 150 μ m mediolaterally. An arrow head shows the point of entrance into the spinal cord. Scale; 100 μ m. **(B)** Photomontage of the fiber termination in the superficial dorsal horn showing en passant and end-terminals of the fiber. Scale; 50 μ m. **(C)** Transverse terminal profiles in lateral lamina I rotated by the computered graphic system. Scale; 100 μ m. **(D)** Diagram showing arborization of the mechanical-cold nociceptor terminals in relatino to boundaries of the dorsal horn laminae.

Fig. 5 A C-mechanical-cold nociceptor fiber in the L6 spinal cord. The receptive field was located on the backside thigh skin. **(A)** Sagittal reconstructed view of the fiber. A descending fiber runs in Lissauer's tract. Terminal arborization extended to 450 μ m rostrocaudally and 150 μ m mediolaterally. An arrowhead indicates the entry into the spinal cord. Scale; 100 μ m. **(B)** Photomontage of the fiber and terminal swellings in the superficial dorsal horn (lamina I). Scale; 50 μ m. **(C)** Transverse drawing of the MCN fiber which terminated at the border of laminae I and II of the dorsal horn.

150 μ m の領域を持っていた。このように吻尾方向に走る分枝を持つ腰髄の終末像は有髄神経の終末分布と類似している^{47,72)}。この MCN の終末膨大の大きさは 10 μ m に達するものもあった (Fig. 5)。

4. 低閾値機械的受容器 (LTM)

LTM は他の 2 つの侵害受容器と異なり無髄神経の中で痛みの伝達に関与しない線維である。この受容体は耳介外側皮膚の軽いタッチに応じた。この LTM の中枢性終末は他の頸髄の終末と同様に後方

Fig. 6 Central projection of a C-low threshold mechanoreceptor in the C2 spinal segment. Receptive field located on lateral skin of pinna responsive to light touch. (A) Sagittal view of the computer reconstructed fiber based on 20 serial sections. The fiber runs caudally in Lissauer's tract and gives off several collaterals to terminate in the lateral part of lamina II. Terminal arborization reveals a narrow, long terminal band extending in 380 μm rostrocaudally and 120 μm mediolaterally. Scale; 100 μm . (B, C) Photomontage and traced drawing of the terminal arborization. Terminal swellings and end terminal branches were seen in the narrow terminal arborization. (D) Rotated transverse view of the computer reconstructed fiber. Several collaterals run the lateral margin of the dorsal horn and formed the narrow terminal arborization. (E) Transverse drawing of the fiber termination relative to the various laminae of the dorsal horn; The terminal arborization was located in the middle portion of lamina II in the lateral dorsal horn.

に走り後角外層縁に沿って2本の側枝を出す。最終的にはすべての側枝はII層の内層、外層の境界部に棍棒上の長い神経叢を作っていた。この終末神経叢の大きさは吻尾方向に380 μm 、内外側に120 μm であった (Fig. 6)。

いずれの感覚タイプの受容器であれC2後根神経節の無髄一次求心性神経の中樞終末は後方に走り、脊髄のI, II層を中心に終末していた。一方、腰髄での無髄神経終末はI, II層に終末神経叢の領域を持つが、脊髄内での走行形態は末梢神経の分布域によって異なると思われた (Fig. 7)。

Fig. 7 Schematic diagrams illustrating the location of physiologically identified unmyelinated fibers from the skin. The transverse plane drawings were reconstructed from distribution observed in parasagittal histological sections of the cervical or the lumbar spinal cord.

30488

Fig. 8 Sagittal view of the reconstructed projection of a visceral afferent C-fiber. The outline of central arborization of the fibers are shown in the camera lucida drawing. On entering the spinal cord (arrowhead in third from the top segment), the axon bifurcates into ascending and descending main branches. The main ascending and descending branches run on the dorsal surface of the spinal cord or Lissauer's tract as far rostrally as the 10th thoracic segment (top left) and as far caudally as the second lumbar (bottom right) segment. Many collaterals leave these parent branches to terminate in laminae I, and II. Some collaterals also terminate in the lateral funiculus and ipsilateral or contralateral laminae V and X. Black stars, open stars and asterisks indicate where the illustrations are interrupted. Circle number indicate the terminal branches drawn in Figure 9 as representative terminals from each layer. LF, Lateral funiculus; V, ipsilateral lamina V; CV, contralateral lamina V; X, ipsilateral lamina X.

内臓からの無髄求心性神経終末

内臓からの無髄神経終末は腹腔神経節近傍の神経の電気刺激により、Th13 後根神経節で細胞内活動電位を得て記録し、標識した。その他の方法は皮膚での記録と同様である。無髄の内臓求心性神経は、電気刺激部位と細胞記録をした Th13 後根神経節との直接距離が短いにもかかわらず、スパイクの伝導時間がかなり延長するものがみられた。これはおそらく一次求心性神経が経由する交感神経節の数、線

維の入る交感神経節のレベルによると考えられた。

骨盤内臓求心性線維を HRP で染色した所見では脊髄後角の内側、外側を通り終末している^{26,42,43,55,56}。単一神経で記録した無髄内臓求心性神経の脊髄終末の分枝は、皮膚などからの体性無髄神経が集中した神経叢を形成するのに対して、全く違った神経分布をしている。求心性線維は後根から脊髄に入り、吻側枝と尾側枝に分岐し、およそ数分節にわたり上行、下行する。そしてその間に 20 本近い側枝を脊髄の I, II, IV, V, X 層、側索、反対側の V, X 層

Fig. 9 Terminal branches from the visceral afferent C-fiber of Figure 8 drawn in parasagittal sections at high magnification. The circled numerals correspond to those in Figure 8. Number 2, 4, 11, and a part of 12 are in lamina I; parts of 3, 8 are in lamina II; 1, 5, 9 and 10 are in lamina V; 7 is in contralateral lamina V; and 6 is in lamina X. The array of terminal branches is arranged with orientation to the neuropil.

に終末する^{54,91)} (Fig. 8). 終末分枝は神経叢を作らず、比較的単純で真っ直ぐな終末分布で1分枝支配領域は狭い⁹²⁾ (Fig. 9).

筋からの無髄 (C または Group IV) 求心性線維

筋からの無髄求心性線維は後肢腓腹筋の外側頭の支配神経を電気刺激し、L5 後根神経節で細胞内ユニット電気記録を行い、伝導速度により無髄神経であることを確認した。筋からの無髄神経の中には神経終末が尾側から吻側に後索表層を数分節にわたり走行するものもあった。神経終末は主に I, II, III 層に終末しており、終末膨大の密度がそれほど高くない神経叢を形成していた。神経叢は全終末分布領域の中に1~2ヵ所に存在し、その領域の広がりには吻尾方向に200~600 μm、内外側に200~300 μmであった。終末膨大は比較的小さく、大きさは最大2~3 μmであった。腓腹筋 C 線維 (group IV) の腰

髄での分節の広がりには他の HRP の注入実験の結果と同じであるが^{51,77)}、脊髄後角における分布は明らかに異なり表層 I, II, III 層に限られていた。

無髄神経の定量的比較

体性神経、内臓神経、筋神経の3種類の無髄神経の定量的検討として、終末分布領域にある終末膨大の数で比較してみると、体性神経は主に I, II 層に神経叢を形成し、1 終末領域に約 700~1500 個の終末膨大を数えた。一方内臓神経は I, II 層に体性神経の約3倍の2500~4000 個の終末膨大を数えるが、終末分枝の数が多く、1 神経終末領域は200~300 個の終末膨大を持つにすぎない (Table 2)。筋神経では I, II 層にある終末膨大は1500~3000 個の終末膨大を数えるが、神経叢の数は2~3 領域にすぎないのでその数は1 領域500~1000 個に相当する^{38,39)} (Table 3)。

Table 2 Number of terminal swellings of visceral and somatic fibers

Case Number	Visceral fibers		Somatic fibers	
	10588	30488	70887	72987
Number of swellings	6099 (100)	5370 (100)	1482	1450
Number of terminal area	22	18	1	2
Mean number of area	277	298	--	725
Dorsal funiculus	359(5.9)	1089(20.3)		
Lamina I	3735(61.2)	2466(45.9)		
lamina II	240(3.9)	151(2.8)		
lamina IV and V	391(6.1)	553(10.3)		
lateral funiculus	276(4.5)	21(0.4)		
Lamina X	171(2.8)	153(2.8)		
Contralateral X	121(2.0)	61(1.1)		
Contralateral IV and V	108(1.8)	142(2.6)		
Total number(%)	5401(88.6)	4636(86.3)		

Table 3 Number of terminal swellings of C-fibers from the gastrocnemius muscle

Case Number	010201	010206	010327
Dorsal funiculus	3261	1068	1660
I abd II	1393	2395	2874
III	53		180
Number of terminal area	3	2	3
Total number	4670	3463	4714

また吻尾方向の領域の広がりを見ると内臓神経は数分節、20 mm にも及ぶが、体性神経はほとんど数百ミクロンに展開するのみである。筋肉からの無髄神経は数分節にわたるものから数百ミクロンに分布するものまで様々であった。

体性無髄神経の脊髄終末の分布領域、すなわち吻尾方向に数百ミクロン、内外側に 200 ミクロンという広がりには後角膠様質の神経細胞の広がりとは驚くほど一致している^{80,89)}。内臓神経の側枝の分布が数百ミクロン間隔であるということは、交感神経の節前神経節細胞の梯子状構造の間隔とほぼ一致している^{32,33,57,60,61,63-65,78)}。また分枝における終末膨大の密度の低さは、内臓感覚あるいは関連痛の局在性のなさ、曖昧さの形態学的背景となるものかもしれない^{10,18,21,22,27,49,59,62,79,81)}。腓腹筋の C 線維 (group IV) の分節の広がりとは脊髄後角における分布は、C 線維の伝える感覚、あるいは末梢受容器の存在する組織の違いを表しているのかもしれない⁵⁰⁾。

現在、痛みの解析が分子レベルで進んでゆく中でも、痛みの原因を見つけ、理解する上では、機能形態学的背景は不可欠なものである。この分野でのさらに詳細な研究が期待される。

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