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Species and strain differences in rodent sciatic nerve anatomy: Implications for studies of neuropathic pain

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Abstract

Hindlimb pain models developed in rats have been transposed to mice, but assumed sciatic nerve neuroanatomic similarities have not been examined. We compared sciatic nerve structural organization in mouse strains (C57BL/6J, DBA/2J, and B6129PF2/J) and rat strains (Wistar, Brown Norway, and Sprague–Dawley). Dissection and retrograde labeling showed mouse sciatic nerve origins predominantly from the third lumbar (L3) and L4 spinal nerves, unlike the L4 and L5 in rats. Proportionate contributions by each level differed significantly between strains in both mice and rats. Whereas all rats had six lumbar vertebrae, variable patterns in mice included mostly five vertebrae in DBA/2J, mostly six vertebrae in C57BL/6J, and a mix in B6129PF2/J. Mice with a short lumbar vertebral column showed a rostral shift in relative contributions to the sciatic nerve by L3 and L4. Ligation of the mouse L4 nerve created hyperalgesia similar to that in rats after L5 ligation, and motor changes were similar after mouse L4 and rat L5 ligation (foot cupping) and after mouse L3 and rat L4 ligation (flexion weakness). Thus, mouse L3 and L4 neural segments are anatomically and functionally homologous with rat L4 and L5 segments. Neuronal changes after distal injury or inflammation should be sought in the mouse L3 and L4 ganglia, and the spinal nerve ligation model in mice should involve ligation of the L4 nerve while L3 remains intact. Strain-dependent variability in segmental contributions to the sciatic nerve may account in part for genetic differences in pain behavior after spinal nerve ligation.

Keywords

Neuropathic pain; Dorsal root ganglion; Pain models; Spinal nerve Ligation; Sciatic nerve anatomy

1. Introduction

A strong motive for the use of mice in the exploration of mechanisms that generate and maintain chronic pain is the availability of genetically-characterized or manipulated inbred strains, particularly transgenic mouse lines in which specific proteins or signal transduction components have been altered through genetic knockout technology. To take advantage of

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these opportunities, pain models originally developed in rats have been transposed for use in mice. These models often involve inflammation, trauma, or tumor implantation in the hind limb, and require examination of the sensory neurons seated in the dorsal root ganglia (DRG) that supply the sciatic nerve with primary afferent fibers. In other cases, peripheral nerves are themselves the target of injury, such as in the chronic constriction injury [2] and the partial sciatic nerve injury [24] models of neuropathic pain. Appropriate analysis of pathologic changes in the somata of the involved sensory neurons requires correct identification of the neuronal segmental levels where the relevant ganglia might be harvested or the relevant spinal nerve might be ligated. Also, the segmental spinal nerves may themselves be the target for selective injury, as in the spinal nerve ligation (SNL) model [14]. Although the relevant anatomy of the rat sciatic and spinal nerves has been extensively investigated [5,11,21,26, 29], there has been no specific examination of the assumed anatomic similarities between mice and rats.

There is known to be significant variability in segmentation of the lumbar vertebral column in mice, both between and within strains [9]. Thus, it is possible that bony landmarks devised for surgical procedures in rats may not be directly applicable in mice without modification. Variability in the number of bony segments also raises the possibility of unrecognized differences in neuronal segmentation between rats and mice, particularly in the contributions to the sciatic nerve. Accordingly, this study was designed to determine the fundamental anatomy of the sciatic nerve in the mouse and the influence of axial lumbar bony segmentation on the level of spinal nerves that contribute to the sciatic nerve.

2. Experimental procedures

2.1. Experimental animals

Adult male mice weighing 20–30 g were examined from three strains. The C57BL/6J (termed C57 hereafter; $n = 66$ total for all protocols; vendor: Jackson Laboratory, Bar Harbor, ME, USA) and the DBA/2J (termed DBA hereafter; $n = 30$; Jackson Laboratory) are widely used inbred strains. We also examined a hybrid strain, B6129PF2/J (termed F2 hereafter; $n = 20$; Jackson Laboratory) in which second filial generation individuals are bred from first filial parents that are a C57BL/6J × 129P3/J cross. Since rats are commonly used in pain studies and were used to develop the most common pain models, additional observations were obtained from three strains of adult rats (250–350 g), specifically Sprague–Dawley (SD, $n = 45$; vendor: Harlan Sprague–Dawley, Indianapolis, IN, US), Wistar ($n = 10$; Harlan Sprague–Dawley), and Brown Norway (BN, $n = 9$; Charles River, Wilmington, MA, USA). All procedures were approved by the Medical College of Wisconsin institutional animal care and use committee.

2.2. Dissection

After animals were euthanized by an overdose of the inhaled anesthetic isoffurane, the skin and viscera were removed. The ribs were counted bilaterally, and the most caudal rib marked with ink. The most craniad vertebra that lacked an articulation with a rib at its rostral margin was denoted as the first lumbar (L1) vertebra, and was also marked. Under 20× magnification, muscle was removed to expose the nerves of the lumbosacral plexus up to the inter-vertebral foramina, taking care to avoid stretching, or compression of the nerves. The sciatic nerve was identified at its departure from the pelvis and tracked back to the plexus, and the components of the anterior divisions of all spinal nerves contributing to the sciatic nerve were identified. The dissected preparation was photographed (pixel size 14μm) from an anterior perspective. Further dissection was then performed to expose the vertebral, sacral, and medial iliac bones, which were cleared of overlying tissue in order to identify the sites of fusion of the lower lumbar vertebrae. Measures of distances between bony structures (detailed below) were performed in

a subset of mice using digital calipers on intact skeletons prepared by boiling, removal of muscle, and tendon, and bleaching with 1% hydrogen peroxide for 10 min.

2.3. Measurement of spinal nerve contributions to the sciatic nerve

The widths of the nerve components contributing to the sciatic nerve were digitally measured in triplicate from the calibrated photographic images (Metamorph 5.0, Molecular Devices Corporation, Sunnyvale, CA) by an observer blinded to the strain of the animal.

In order to convert nerve width into an axial section area without assuming a specific geometry of the cross-section, we fixed the entire dissected preparation of a representative subset of animals ($n = 5$) in 4% paraformaldehyde after photography. The measured portions of the spinal nerves were excised and embedded in mounting medium (Tissue-Tek OCT Compound, Sakura Finetek, Torrence, CA) and cryo-sectioned (15μm thickness). Tissue sections were photographed microscopically (E600 microscope, Nikon USA, Melville, NY, USA; Spot CCD camera, Diagnostic Instruments, Sterling Heights, MI, USA), and the cross-sectional areas of these nerves were determined digitally (MetaMorph 5.0). An exponential function provided a close fit ($R^2 = 0.91$) of the plot of microphotographic width *vs*. area measured in the preserved tissue cross-sections (Fig. 1), permitting calculation of an estimated axial section area for the dissected nerves. These data indicate that photographic measurement of the width of the nerve provides a valid estimate of nerve area. Although fixation may alter the nerve shape, the area of the nerve should not be affected except by shrinkage, which would affect all area measurements proportionately, so that this calculated area is representative of proportionate neuronal content.

2.4. Lumbar segmental levels of neurons projecting to paw and sciatic nerve

We further determined the segmental levels of sensory neurons that project to the plantar surface of the hind paw, where pain testing is often performed, and to the sciatic nerve in the thigh, where various inflammatory and traumatic injuries are imposed. True Blue dye (15 μL, 1% solution in PBS; Sigma–Aldrich, St. Louis, MO, USA) was injected subcutaneously in the center of the plantar aspect of the paw just proximal to the plantar tori, through a 29 g needle during brief isoflurane anesthesia. Sciatic nerve injections of True Blue dye were performed in a separate group of mice. During anesthesia, a lateral skin incision was made to expose the sciatic nerve at the mid-thigh, and 2 μL of dye solution was injected subepineurially through a calibrated glass micropipette (40 μm tip diameter) stabilized in a micromanipulator, using repeat air pulses (20 ms at 15 psi; Picospritzer II, General Valve Corp., Fairfield, NJ) over 1 min. Microscopic examination (l0×) assured that dye leakage was avoided, the field beneath the nerve was protected by a foil barrier, and the field was generously irrigated at conclusion of the injection.

Six ipsilateral DRGs, beginning with LI and moving caudally, plus one contralateral DRG, were harvested 5–6 days after sciatic nerve injection and 7–8 days after paw injection. Following fixation in 4% paraformaldehyde and overnight equilibration in 30% sucrose, DRG tissue was sectioned (35μm thick) in random orientation, and the complete section series through each DRG was examined. Sections were examined with UV filters for True Blue (excitation 330–380 nm, emission 435–485) and fluorescent images were made using identical illumination and acquisition parameters throughout all images for each animal. All True Blue-positive neurons were counted throughout all tissue sections from each DRG (15.5 ± 4.5 sections per DRG). Counting was performed by an observer who was blinded to mouse strain and level. For each DRG section series, a threshold was established for image intensity above which a neuron was considered positive. Specifically, images from the contralateral DRG were referenced, as no True Blue-positive neurons were ever observed in any contralateral ganglia and these images were comparable to sections from non-injected animals. The threshold for

each image from the ipsilateral ganglia was set as twice the brightness value of the brightest pixels in the contralateral ganglia. There was minimal autofluorescence.

2.5. Motor and sensory behavioral evaluation

Specific nerve ligation at various lumbar levels was performed during anesthesia in mice and rats, using a modification [12] of the original SNL technique [14]. Post-operatively, motor and sensory evaluation of the hind limbs was performed by an observer blinded to the nature of the injury. The ipsilateral and contralateral hind limb postures and use were examined during natural ambulation in an open area. Hyperalgesia was tested using a technique that selectively identifies animals with neuropathic pain behavior [12]. Specifically, touching the foot by a pin with pressure adequate to indent but not penetrate the skin produces either a brief reflexive flinch withdrawal of the foot or a complex hyperalgesia maneuver involving sustained lifting, grooming, shaking, or stomping of the foot. Response type was tabulated during ten touches alternately applied to the ipsilateral and contralateral plantar surface before injury and on 3 different days after injury. Mice were tested up to 7 days after injury and rats up to 18 days after injury on the basis of previous experience with SNL in these species [12,20].

2.6. Statistical analysis

Initial analysis showed that right and left nerve areas behaved independently. Specifically, comparing estimated nerve area for the right and left side at each level for each mouse showed that the absolute value of the difference between sides was 21–25% of the average area. We thus considered the right and left sides as separate data. Since there were no significant differences in average measures grouped by right *vs*. left, the data were pooled for further calculations. A repeated measures ANOVA model (segmental level as within factor; strain or number of vertebrae as a categorical factor) was used to compare groups (Statistica 6.0, StatSoft, Tulsa, OK). Actual data (calculated nerve area or positive neuron counts) were used, although figures show percentile contributions. Planned comparisons between groups were performed by one-way ANOVA at each level and Bonferroni post hoc comparisons. Sensory behavior was analyzed by repeated measures ANOVA with two within factors, side (ipsilateral *vs*. contralateral) and time (days post-surgery). Cross-tabulation and Pearson's x^2 were used for non-parametric analysis of bony segmentation, inter-segmental neuronal connections, and motor behavioral features. Significance levels were set at $P < 0.05$. Data are presented as means ± SD.

3. Results

3.1. Observations from gross dissection

3.1.1. Mice—All mice had 13 ribs bilaterally with the exception of one F2 animal in which there were only 12 on the right side and a vestigial small 13th rib on the left. In all three strains of mice, the sciatic nerve was composed of elements originating from the L3 and L4 spinal nerves with a smaller diameter contribution from L5 (Fig. 2A and B). There were inconsistent (116/148, 78%), strain-dependent connections between the L2 and L3 spinal nerves (Table 1) that in most cases (104/116, 90%) consisted of a branch from the L3 nerve diverging to join the L2, thus contributing to the obturator and femoral nerves. In the other cases, these nerves joined in such a way that the direction of fiber passage was indiscriminate. In all cases (n = 152), there was a complete merging of the L3 and L4 spinal nerves. In 143/150 (95%) of cases, there was an L5 segmental contribution to the sciatic nerve. However, its size and proportional contribution to the sciatic nerve were only evident when the pudendal nerve was pulled away from the sciatic nerve, which readily resulted in separation of the apposed nerves, whereupon the actual contributing branch could be identified (Fig. 2C). There were no visible contributions from the L6 spinal nerve to the sciatic nerve in mice.

3.1.2. Rats—All rats had 13 ribs, and in all cases the sciatic nerve was composed predominantly of elements originating at levels L4 and L5 (Fig. 2D). This confirms previous observations that 98–99% of the somata of primary afferent neurons projecting to the sciatic nerve reside in the L4 and L5 DRGs in rats [29]. Connections of these components with L3 were small and were strain-dependent (Table 1). Likewise, the contribution of L6 was variable between strains, ranging from no connection to full incorporation into the sciatic nerve, confirming previous observations [1].

Comparison of the mouse and rat patterns (Table 1) shows that in mice only L3 and L4 spinal nerves always join and always contribute to the sciatic nerve, as is true of the L4 and L5 nerves in rats, supporting the homology of these nerve levels between the two species.

3.2. Quantification of spinal nerve segmental contributions to the sciatic nerve

The proportionate contribution of specific spinal nerves to the sciatic nerve was determined from photographically measured diameters, converted into estimated cross-sectional areas.

3.2.1. Mice—In mice, the main effect of strain on nerve area showed a trend only ($P = 0.058$), but spinal segmental level strongly affected area of the nerves contributing to the sciatic nerve ($P < 0.0001$). Quantification (Fig. 3) shows that in all strains of mice examined, the L3 and L4 spinal nerves provide the primary contribution to the sciatic nerve. There was a highly significant interaction between strain and segmentation ($P < 0.0001$), indicating that the influence of segmental level on areas of nerves contributing to the sciatic nerve differs between strains. This is evident from the dissimilar proportionate contributions of L3 and L4 in the different strains (Fig. 3A). Specifically, for DBA and F2 mice, the L3 and L4 spinal nerve contribution is relatively equal, whereas in the C57 strain, the L4 contribution is greater than the L3 contribution. In comparison, the L5 spinal nerve gives a proportionately smaller contribution in all three mouse strains, and the L6 spinal nerve does not contribute at all.

3.2.2. Rats—In rats as in mice, there was no main effect of strain on nerve area (Fig. 3B, $P = 0.357$), and spinal segmental level strongly affected area of the nerves contributing to the sciatic nerve ($P < 0.0001$). In contrast to mice, however, the L4 and L5 spinal nerves provide the primary contribution to the sciatic nerve for all three rat strains. This confirms prior observations of the dominance of the L4 and L5 segments in forming the sciatic nerve in the rat [6,29]. There was a significant interaction between strain and segmentation ($P < 0.001$) in areas of spinal nerves contributing to the sciatic nerve. Specifically, Wistar rats show an equal contribution by L4 and L5 spinal nerves, whereas SD and BN rats exhibit a disproportionate contribution from the L5 nerve compared to the L4 nerve. The L6 nerve gives a much smaller contribution to the sciatic nerve (only 5–10% of that of the L5 nerve), but this is highly variable, as some rats show no contribution from the L6 spinal nerve.

3.3. Effect of bony segmentation on composition of the sciatic nerve

Consideration was next given to the possibility that variability in bony segmentation of the vertebral column may influence neuronal contributions to the sciatic nerve.

3.3.1. Mice—In mice, skeletal analysis showed substantial variation ($P < 0.001$) between strains with respect to the number of lumbar vertebrae (Fig. 4, and Table 2). In the C57 strain, the majority (86%) of animals had six symmetric lumbar vertebrae (conventionally termed 6/6 for left/ right). In contrast, almost all (96%) DBA animals had only five symmetric lumbar vertebrae (5/5 pattern). The hybrid F2 strain showed a mix of both patterns, in which 60% had a 6/6 pattern and 30% had a 5/5 pattern. Variable fusion of the sixth infra-thoracic vertebra determines whether it appears as a sixth lumbar or first sacral element. This was clearly

demonstrated by the occurrence of some mice in each strain that exhibited fusion asymmetry, with a 5/6 ($n = 3$) or 6/5 ($n = 4$) pattern (Table 2, and Fig. 4B).

Specific comparisons were made to identify whether bony segmentation influences the pattern of spinal nerve contributions to the sciatic nerve. A contrasting balance of L3 and L4 was identified when comparing all 5/5 animals with all 6/6 animals ($P < 0.0001$ for interaction between number of vertebrae and nerve level effects), in which the presence of more (six instead of five) lumbar vertebrae was associated with a caudal shift in contributions to the sciatic nerve, such that L4 rather than L3 predominated (Fig. 5A). When 5/5 and 6/6 animals of only the F2 strain were compared, a trend for a similar caudal shift was observed ($P = 0.062$, Fig. 5B), with somewhat greater proportional contribution by L4 in 6/6 animals. Comparing the fused (five free transverse processes) and non-fused (six free transverse processes) sides of asymmetric animals revealed no influence on neural pattern (Fig. 5C). These data suggest that the presence of five *vs*. six lumbar vertebrae in mice may affect the balance of contributions by the specific spinal nerves to the sciatic nerve.

3.3.2. Rats—All three common rat strains examined consistently had six lumbar vertebrae (Table 2). The finding that lumbar bony segmentation is stable across strains in rats is further supported by the fact that we found no rats with asymmetric lumbar fusion. However, we nonetheless observed significantly different patterns of spinal nerve contributions to the sciatic nerve in different rat strains ($P < 0.001$, Fig 3B). This finding indicates that there are factors other than bony segmentation that influence the relative contributions of lumbar spinal nerves to the sciatic nerve in rats.

3.4. DRG neuronal somata projecting to paw and distal sciatic nerve

The plantar skin and the sciatic nerve in the thigh are sites that have particular relevance to rodent pain research, but the relative contributions of different lumbar DRG levels to the sciatic nerve have not been examined in the mouse. One report [27] identified sciatic-projecting neurons in the L5 DRG of C57 mice, but other DRG levels were not examined to determine the relative composition of the spinal origins of the sciatic nerve. We considered using a stereological approach for counting somata in the DRGs at different levels. However, this would not distinguish the neurons at those levels that project to the sciatic nerve, and therefore could not address our goal of learning which ganglia house neurons project to the sciatic nerve and what proportion of sciatic neurons they represent. We also considered performing fiber counts in the spinal nerve components contributing to the sciatic nerve. However, there is a significant contribution of motor fibers to the sciatic nerve, and this approach could not distinguish afferent sensory fibers from efferent motor fibers.

Therefore, we employed a retrograde labeling strategy in order to determine the relative segmental distribution of neurons with sensory fibers projecting to the hind paw and the sciatic nerve in the thigh. We used True Blue dye, which reliably labels only DRG neurons after peripheral injection [10]. We used subcutaneous injection for labeling neurons innervating the plantar aspect of the foot and a subepineurial microinjection technique for labeling sciatic-projecting neurons. These methods label a sample population but not the total neuronal population, and thus provide ratio data that are adequate to resolve the relative roles of the different segments contributing to the sciatic nerve. Although exposure of sectioned ends of peripheral nerves to the label provides more complete neuronal staining [29], this method is nonetheless incomplete, and sciatic nerve section in mice induces a substantial neuronal loss in the DRG [27].

We examined DBA and C57 mice, as these differ the greatest in the balance of segmental contributions to the sciatic nerve (Fig. 3A). The dye was readily transported to the lumbar DRGs (Fig. 6), where a large number of positive neurons were evident (average total count

was 1458 ± 1018 after paw injection, and 4715 ± 1734 after sciatic nerve injection). Our results show that when the axons passing either through the sciatic nerve (Fig. 7A) or to the distal receptive fields in the central plantar paw (Fig. 7B) are labeled with a retrograde tracer, the L3 and L4 DRGs are the ganglia that contain the majority of True Blue-labeled somata in both DBA and C57 mice. These findings confirm our observation from photographic nerve measurement that the sciatic nerve in mice is composed predominantly of fibers originating at the L3 and L4 DRG levels.

The distribution of True Blue-labeled sensory neurons after paw injection also confirms a distinction between mouse strains in the proportional segmental origins of the sciatic nerve. Specifically, a significant main effect ($P < 0.001$) for the interaction between strain and segment on positive cell counts was evident by ANOVA, due to relatively diminished neuronal counts in more distal segments in DBA animals compared to C57 mice, with a compensatory increase in L3 positive neurons (Fig. 7B). Since all injected C57 animals had a 6/6 pattern of lumbar bony segmentation while DBA animals all had a 5/5 pattern, the shift in the balance of paw and distal sciatic sensory fibers to more rostral origins in the DBA animals may represent an influence of bony segmentation on neuronal distribution. Thus, the tracer and dissection methods show very similar patterns in the strains we tested, despite the fact that the tracer labeled only sensory axons, and identified only the subset of the sciatic fibers that projected to these distal sites.

3.5. Behavioral determination of spinal nerve roles

Although extensive behavioral analysis is beyond the scope of this anatomic study, we examined behavior in C57 mice and SD rats after ligation and section of selected spinal nerves in order to test the possible functional homology of segmental levels that thus far have shown anatomic homology. Using a test for hyperalgesia in response to noxious mechanical stimulation of the plantar paw [12], we observed ipsilateral mechanical hyperalgesia in mice following L4 spinal nerve ligation ($P < 0.001$, $n = 11$) that was absent after sham L4 ligations ($n = 5$; Fig. 8). We additionally confirmed the development of mechanical allodynia 2 days after mouse L4 ligation by testing with von Frey fibers [3] (baseline withdrawal threshold of 0.13 ± 0.15 g; contralateral to L4 ligation 0.13 ± 0.12 g; ipsilateral to L4 ligation 0.03 ± 0.03 g, $P < 0.001$). Attempted L5 ligation in mice produced substantial tissue damage due to the relative inaccessibility of this relatively small spinal nerve anterior to the pelvis, and was abandoned. Rat L5 spinal nerve ligation, similar to mouse L4 ligation, produced hyperalgesia ($P < 0.001$, $n = 10$) that was absent in sham controls ($n = 10$; Fig. 8). Ligation of the mouse L3 ($n = 13$) and rat L4 ($n = 9$) spinal nerves produced conspicuous flexor weakness that was selective for these levels (mouse 69% after L3 ligation *vs*. 8% after L4 *vs*. 0% after sham, $P < 0.01$; rat 100% after L4 ligation *vs*. 0% after L5 ligation *vs*. 0% after sham, $P < 0.001$). Reciprocally, cupping of the foot was seen often after mouse L4 ligation (83%) and rat L5 ligation (80%), but rarely after mouse L3 ligation (8%, $P < 0.001$ *vs*. mouse L4) and never after rat L4 ligation ($P < 0.001$ *vs*. rat L5). Thus, the functional roles for L3 and L4 spinal nerves in mice are matched in parallel by L4 and L5 spinal nerves in rats, supporting the anatomic observations from dissection and tracer studies described above.

3.6. Distances between bony landmarks

The variability in the number of mouse lumbar vertebrae we have observed in this study raises a technical challenge for investigators to correctly identify segmental levels, for example, during ligation of a specific spinal nerve in a survival surgery or for accurate harvest of a specific DRG. Since the last rib is not easily identified without extensive surgical exposure, we examined different anatomical landmarks in reference to the iliac crest in order to determine which could reliably identify the segmental level in the absence of knowledge of the number of lumbar vertebrae. Accordingly, we determined distances (Fig. 9) from the rostral edge of

the iliac crest to the rostral edges of the transverse processes (IC-TP dimension) and the superior articular processes (IC-SAP) bilaterally for lower lumbar vertebrae in prepared skeletons of C57 mice (n = 11) and F2 mice (n = 20). All measures were made from the dorsal aspect of the skeleton, in a position similar to that used during most surgical preparations on live mice. As there were no differences in dimensions between left and right, between symmetric and asymmetric designs, or between strains, these data were considered as one group for analysis. Following measurement of these dimensions, the vertebral segmentation pattern was enumerated in reference to the ribs as described above (C57: 2 asymmetric, the others 6/6; F2 mice: two asymmetric, six symmetric with 5/5 vertebrae, 12 symmetric with 6/6 vertebrae).

On average, significantly different IC-TP distances were found comparing the L5 and L6 transverse processes on sides with six lumbar vertebrae and the L5 transverse process in animals with five lumbar vertebrae (Fig. 10A). However, there was an overlapping distribution of dimensions, so it would be impossible to reliably identify a transverse process 1.0–1.5 mm rostral to the iliac crest without knowing the number of lumbar vertebrae. In contrast, not only did IC-SAP distances show significant differences in their averages, but also there was no overlap between the IC-SAP distance for the L6 articular process in animals with six lumbar vertebrae and for the L5 articular process in animals with five lumbar vertebrae, even when fusion was asymmetric. Thus, the first superior articular process more than 1 mm rostral to the iliac crest can reliably be identified as the L5 in all mice in our data set, regardless of segmentation pattern, so this landmark may guide mouse dissection for tissue harvest and injury models.

4. Discussion

4.1. Different segmental contributions to the sciatic nerve in rat and mouse

The rat sciatic nerve receives 98% of its sensory fibers from the L4 and L5 spinal nerves [29]. In contrast, the sciatic nerve of the three strains of mice examined in this study uniformly received fibers predominantly from L3 and L4 spinal nerves. Therefore, the result of distal hind limb inflammation, cancer, or sciatic nerve injury in mice must be sought in the L3 and L4 neuronal segments. Performance of the SNL model in mice should entail ligation of the L4 spinal nerve and possibly the L5 (although we found this nerve to be relatively inaccessible from a posterior approach), while axotomy-induced changes in neighboring, intact neurons will be evident in the adjacent L3 DRG.

4.2. Bony segmentation of the lumbar vertebral column is variable in mice

Variable fusion at the lumbosacral junction in mice is genetically regulated [7,9], as demonstrated by interstrain variability of presacral segmentation in our data. There is additional variation due to gender (males have fewer lumbar vertebrae [9,17,23]) and to *in utero* factors such as maternal age and diet [4,9,18,23]. Asymmetric fusion is more common on the right than on the left [9,17,23]. In contrast to mice, all rats of three different strains showed a 6/6 pattern of lumbar vertebrae, which demonstrates a highly consistent bony segmentation of the rat lumbar vertebral column.

4.3. Segmental composition of the sciatic nerve is variable between strains

The smallest segmental source of the sciatic nerve was always the most caudal of the three spinal nerve origins (L5 in mice, L6 in rats). There was variability between strains, however, in the proportions provided by different levels. The dominant root of the sciatic nerve may even differ between strains, as L4 is largest in C57 and F2 mice, whereas L3 is somewhat larger than L4 in DBA mice. This inter-strain effect may in part be attributable to the shorter lumbar vertebral column in C57 strain compared to DBA mice, since a rostral shift in the bony lumbosacral junction is associated with a comparable rostral shift in the origin of neuronal

origins in humans [13,25,32] and mice [16]. However, we also discovered large inter-strain differences in proportionate contributions to the sciatic nerve in rats, which all shared a common 6/6 bony pattern. Clearly, neural pattern is only partially regulated by bony structure.

4.4. Consideration of the published literature in the context of the present findings

Our findings raise a critical issue of how to interpret the many prior studies in which the L4/L5 dominant composition of the sciatic nerve in rats was incorrectly assumed to hold true in mice. Several types of studies are involved. Those that examine effects of spinal nerve axotomy on DRG somata are unlikely to be influenced by incorrect level identification since tissue harvest will almost certainly be performed at the same level as the axotomy, either through identification of prior operative changes at the time of harvest, or by employing the same landmarks for both procedures.

A greater problem arises in mouse studies that have investigated primary afferent somata after inflammation or injury at more peripheral sites such as the paw or sciatic nerve in the thigh. These reports have sought changes in the L4 and L5 DRGs, not L3 and L4 as our present data indicate. Several considerations determine whether our present findings should influence the interpretation of those reports. Firstly, could the mice examined in such reports differ in anatomic pattern from those used in the present study? Against this possibility, we found an L3/L4 dominant sciatic composition in three different mouse strains, and found no specimens in which L5 provided a dominant contribution to the sciatic nerve. Secondly, would the results differ using the rong pair of ganglia? Since the L4 DRG will correctly reflect peripheral sciatic-mediated effects, the error in tissue harvest is only partial. Since data from minimally affected L5 ganglia are admixed with correct L4 data, incorrect tissue harvest may not be apparent, and reported findings may in fact underestimate actual changes. Thirdly, could the present findings be wrong, and the techniques employed here have led to incorrect identification of mouse lumbar segments contributing to the sciatic nerve? Accepting our data indicates that mistaken anatomical designations have been made in many studies and at different centers, but we suspect that this is in fact the case. We are unaware of a means of misinterpreting the photographic data, and typical examples are provided in the figures. (The complete data set is available by request.) Retrograde labeling confirms the dimensional measurements, and behavioral data show homology of the mouse L3 and L4 nerves with the rat L4 and L5 nerves. There is one report [15] in which an example is shown that concur with our determination. Fourthly, might the other investigators have correctly harvested the L3 and L4 DRGs but used mistaken nomenclature on the basis of the rat precedent? This may happen if the sciatic nerve is followed proximally and the proximal origins are assumed to be L4 and L5 without confirming this, which requires numerical identification of the vertebrae in reference to the placement of the ribs. While we suspect that this is indeed a likely feature of many publications, there are no means of retrospectively weighing these various explanations.

A final setting in which identification of lumbar nerve levels may critically influence research findings is performance of the SNL model. Published studies in mice have described pain behavior after ligation of the L5 spinal nerve, with [8,30] or without [20,22,28,33] ligation of the L6 nerve. Our finding of only a minor contribution of the mouse L5 spinal nerve to the sciatic nerve predicts that L5 ligation will minimally affect the population of afferents going to the foot or sciatic nerve in the thigh. Potential explanations for these divergent claims are similar to those enumerated above. The correct ligation (L4) may have been performed with the wrong nomenclature. This possibility is supported by our observation that the actual L5 spinal nerve is very difficult to expose and ligate in the mouse, in contrast to the more rostral lumbar levels. Alternatively, the incorrect (L5) spinal nerve may have been ligated in these studies, analogous to an exclusive ligation of L6 in rats, for which plantar sensory changes are unknown but likely minor.

4.5. Implications of strain differences in sciatic nerve composition

Inbred mice strains have been shown to differ in the degree of thermal and mechanical hypersensitivity that follow L5 SNL [20]. One reason may be inter-strain differences in the contribution of L5 to the mouse sciatic nerve, such that the degree of injury following SNL done this way will differ accordingly. Alternatively, if the L4 nerve was correctly ligated and only the numerical nomenclature was incorrect, a genetic effect may be due to a variable extent of injury due to different contributions of the L4 spinal nerve to the sciatic nerve and the variable proportion of surviving fibers in L3. We also found inter-strain differences in the proportionate composition of the sciatic nerve in rats, which may in part account for dissimilar behavioral responses to SNL in different rat strains [31].

4.6. Technical considerations for identifying lumbar vertebral level in mice

Variability in segmentation of the mouse lumbar vertebral column creates shifting landmarks for correctly performing selective spinal nerve injury and for harvesting tissue at specific neuronal levels. Indeed, since differences between strains in the number of lumbar vertebrae may result in ligation of different spinal nerves, genetically determined variability in lumbosacral bony segmentation may be an important factor contributing to different degrees of neuropathic pain after anatomically targeted spinal nerve injury in various strains [19,20], if this resulted in ligation of different spinal nerves.

The sciatic nerve branching pattern is an unreliable means of identifying levels since this is inconsistent. Also, without knowledge of the number of lumbar vertebrae, we found that it is not possible to uniquely specify the level of a transverse process. However, the first superior articular process more than 1 mm above the iliac crest can reliably be identified as the L5, regardless of the number of lumbar vertebrae. In all cases, necropsy determination of levels in reference to the last rib is necessary for certain confirmation.

5. Conclusion

The mouse sciatic nerve is composed of contributions predominantly from the L3 and L4 spinal nerves, with a smaller component from the L5 segment. Variable segmentation of the mouse lumbar vertebral column may complicate the correct harvest of neural tissue or accurate performance of the SNL model. The relative proportions of contributions to the sciatic nerve from the L3 and L4 spinal nerves in mice, and from the L4 and L5 nerves in rats, are genetically regulated and could result in different behavioral outcomes even after correctly performed SNL. For these reasons, evaluation of the anatomical substrate will be important for correct interpretation of future studies using hind limb pain models.

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References

1. Asato F, Butler M, Blomberg H, Gordh T. Variation in rat sciatic nerve anatomy: implications for a rat model of neuropathic pain. J Peripher Nerv Syst 2000;5:19–21. [PubMed: 10780679]
2. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988;33:87–107. [PubMed: 2837713]
3. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53:55–63. [PubMed: 7990513]

4. Deol MS, Truslove GM. Genetical studies on the skeleton of the mouse: XX. Maternal physiology and variation in the skeleton of C57BL mice. J Genet 1957;55:288–312.
5. Devor M, Govrin-Lippmann R. Neurogenesis in adult rat dorsal root ganglia. Neurosci Lett 1985;61:189–194. [PubMed: 4080254]
6. Devor M, Govrin-Lippmann R, Frank I, Raber P. Proliferation of primary sensory neurons in adult rat dorsal root ganglion and the kinetics of retrograde cell loss after sciatic nerve section. Somatosens Res 1985;3:139–167. [PubMed: 3835669]
7. Gad JM, Tam PP. Axis development: the mouse becomes a dachshund. Curr Biol 1999;9:R783–R786. [PubMed: 10531023]
8. Gardell LR, Ibrahim M, Wang R, Wang Z, Ossipov MH, Malan TP Jr, et al. Mouse strains that lack spinal dynorphin upregulation after peripheral nerve injury do not develop neuropathic pain. Neuroscience 2004;123:43–52. [PubMed: 14667440]
9. Green EL. Genetic and non-genetic factors which influence the type of the skeleton in an inbred strain of mice. Genetics 1941;26:192–222. [PubMed: 17247002]
10. Haase P, Payne JN. Comparison of the efficiencies of true blue and diamidino yellow as retrograde tracers in the peripheral motor system. J Neurosci Methods 1990;35:175–183. [PubMed: 2283887]
11. Himes BT, Tessler A. Death of some dorsal root ganglion neurons and plasticity of others following sciatic nerve section in adult and neonatal rats. J Comp Neurol 1989;284:215–230. [PubMed: 2474003]
12. Hogan Q, Sapunar D, Modric-Jednacak K, McCallum JB. Detection of neuropathic pain in a rat model of peripheral nerve injury. Anesthesiology 2004;101:476–487. [PubMed: 15277932]
13. Horowitz MT. The anatomy of the lumbosacral nerve plexus – its relation to variations of vertebral segmentation, and the posterior sacral nerve plexus. Anat Rec 1939;74:91–107.
14. Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 1992;50:355–363. [PubMed: 1333581]
15. Malin SA, Davis BM, Molliver DC. Production of dissociated sensory neuron cultures and considerations for their use in studying neuronal function and plasticity. Nat Protoc 2007;2:152–160. [PubMed: 17401349]
16. McHanwell S, Biscoe TJ. The localization of motoneurons supplying the hindlimb muscles of the mouse. Philos Trans R Soc Lond B Biol Sci 1981;293:477–508. [PubMed: 6115428]
17. McLaren A, Michie D. Factors affecting vertebral variation in mice: 1. Variation within an inbred strain. J Embryol Exp Morphol 1954;2:149–160.
18. McLaren A, Michie D. Factors affecting vertebral variation in mice. 4. Experimental proof of the uterine basis of a maternal effect. J Embryol Exp Morphol 1958;6:645–659. [PubMed: 13611143]
19. Minert A, Gabay E, Dominguez C, Wiesenfeld-Hallin Z, Devor M. Spontaneous pain following spinal nerve injury in mice. Exp Neurol. 2007
20. Mogil JS, Wilson SG, Bon K, Lee SE, Chung K, Raber P, et al. Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. Pain 1999;80:67–82. [PubMed: 10204719]
21. Puigdellivol-Sanchez A, Forcada-Calvet P, Prats-Galino A, Molander C. Contribution of femoral and proximal sciatic nerve branches to the sensory innervation of hindlimb digits in the rat. Anat Rec 2000;260:180–188. [PubMed: 10993954]
22. Ramer MS, Murphy PG, Richardson PM, Bisby MA. Spinal nerve lesion-induced mechanoallodynia and adrenergic sprouting in sensory ganglia are attenuated in interleukin-6 knockout mice. Pain 1998;78:115–121. [PubMed: 9839821]
23. Searle AG. Genetical studies of the skeleton of the mouse: IX. Causes of skeletal variation within pure lines. J Genet 1954;52:68–102.
24. Seltzer Z, Dubner R, Shir Y. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. Pain 1990;43:205–218. [PubMed: 1982347]
25. Seyfert S. Dermatome variations in patients with transitional vertebrae. J Neurol Neurosurg Psychiatry 1997;63:801–803. [PubMed: 9416822]
26. Shehab SA, Atkinson ME, Payne JN. The origins of the sciatic nerve and changes in neuropeptides after axotomy: a double labelling study using retrograde transport of true blue and vasoactive intestinal polypeptide immunohistochemistry. Brain Res 1986;376:180–185. [PubMed: 3719366]

27. Shi TJ, Tandrup T, Bergman E, Xu ZQ, Ulfhake B, Hokfelt T. Effect of peripheral nerve injury on dorsal root ganglion neurons in the C57 BL/6J mouse: marked changes both in cell numbers and neuropeptide expression. Neuroscience 2001;105:249–263. [PubMed: 11483316]

28. Shim B, Ringkamp M, Lambrinos GL, Hartke TV, Griffin JW, Meyer RA. Activity-dependent slowing of conduction velocity in uninjured L4 C fibers increases after an L5 spinal nerve injury in the rat. Pain 2007;128:40–51. [PubMed: 17030437]

29. Swett JE, Torigoe Y, Elie VR, Bourassa CM, Miller PG. Sensory neurons of the rat sciatic nerve. Exp Neurol 1991;114:82–103. [PubMed: 1915738]

30. Wang Z, Gardell LR, Ossipov MH, Vanderah TW, Brennan MB, Hochgeschwender U. Pronociceptive actions of dynorphin maintain chronic neuropathic pain. J Neurosci 2001;21:1779–1786. [PubMed: 11222667]

31. Yoon YW, Lee DH, Lee BH, Chung K, Chung JM. Different strains and substrains of rats show different levels of neuropathic pain behaviors. Exp Brain Res 1999;129:167–171. [PubMed: 10591890]

32. Young A, Getty J, Jackson A, Kirwan E, Sullivan M, Parry CW. Variations in the pattern of muscle innervation by the L5 and S1 nerve roots. Spine 1983;8:616–624. [PubMed: 6648707]

33. Zwick M, Molliver DC, Lindsay J, Fairbanks CA, Sengoku T, Albers KM, et al. Transgenic mice possessing increased numbers of nociceptors do not exhibit increased behavioral sensitivity in models of inflammatory and neuropathic pain. Pain 2003;106:491–500. [PubMed: 14659533]

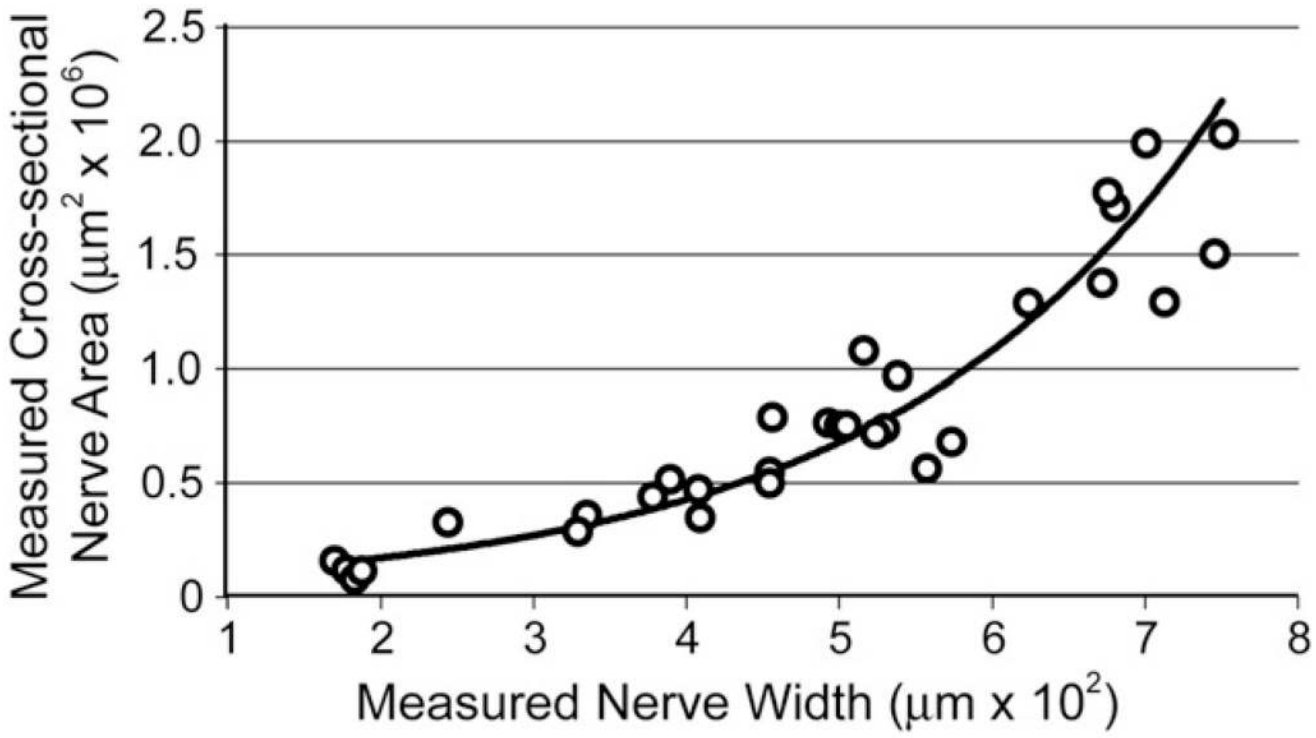

Fig. 1.
Comparison of nerve diameter measured photographically from the dissected mouse after fixation and nerve cross-sectional area after excision and microscopic measurement. The line shows a fit by the equation $y = 6442e^{0.0047x}$ with $R^2 = 0.91$

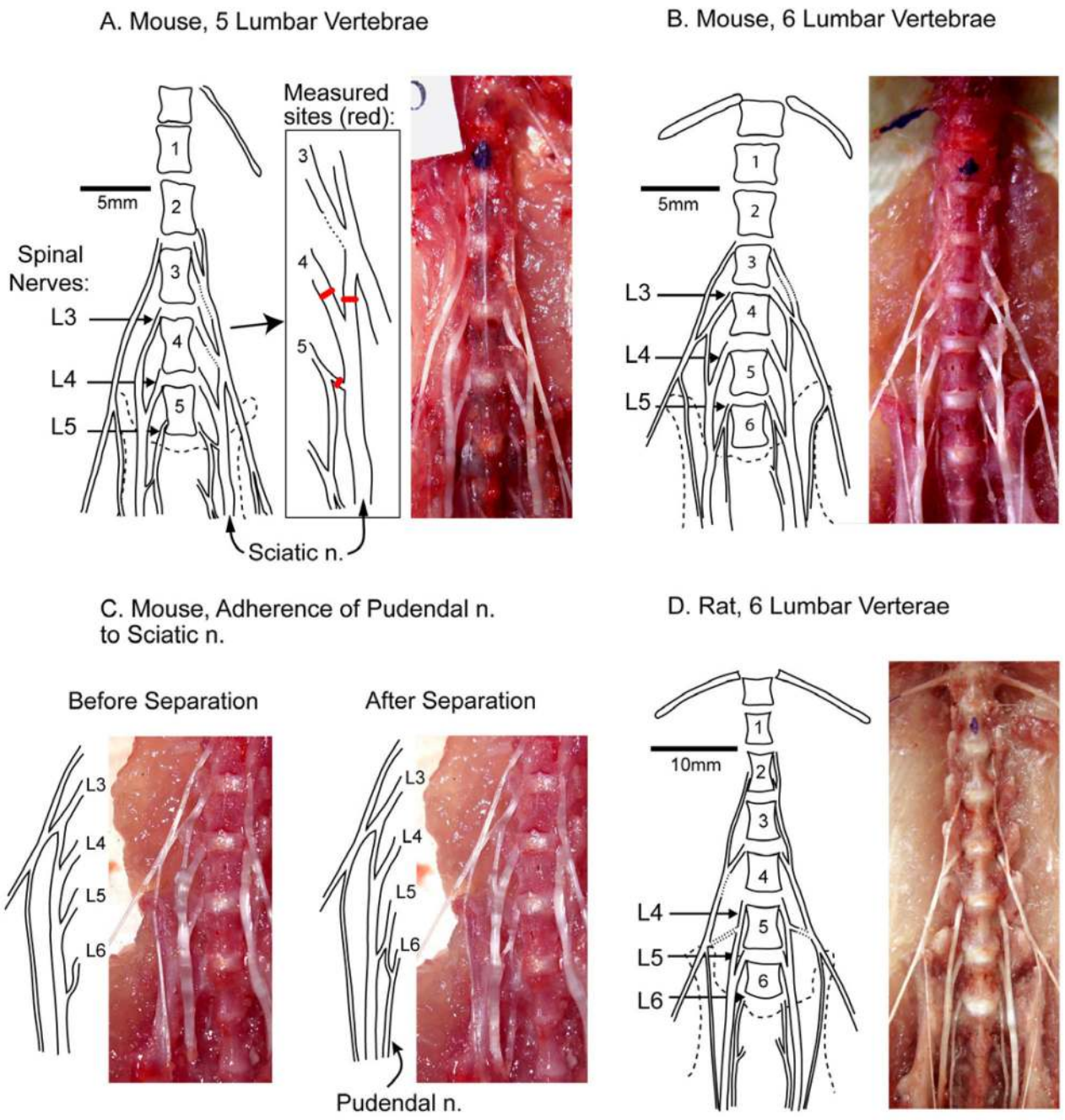

Fig. 2.

Ventral views of dissections and reference drawings made from them, showing the sciatic nerve and its segmental origins. (A) DBA/2J strain mouse with five lumbar bony segments bilaterally. The third lumbar (L3) spinal nerve, together with the L4 and L5 (arrows), contributes to the sciatic nerve. The inset line drawing shows the sites of diameter measurement on the anatomic left side (enlarged). (B) C57BL/6J strain mouse with six lumbar bony segments bilaterally. As with mice with five lumbar vertebrae, the L3, L4, and L5 spinal nerves (arrows) make up the sciatic nerve. (C) In mice, the L5 spinal nerve may appear to make a large contribution ("Before Separation"), but gentle traction pulls the pudendal nerve (arrow) away from the sciatic nerve to expose a typically slight contribution from the L5 spinal nerve to the sciatic nerve ("After

Separation"). Dotted lines represent nerves hidden by overlying bone. Dashed lines represent the pelvic bones. (D) Sprague–Dawley strain rat with six lumbar vertebrae. In contrast to the mice, the sciatic nerve is composed of L4 and L5. In other rats, L6 may also contribute to the sciatic nerve.

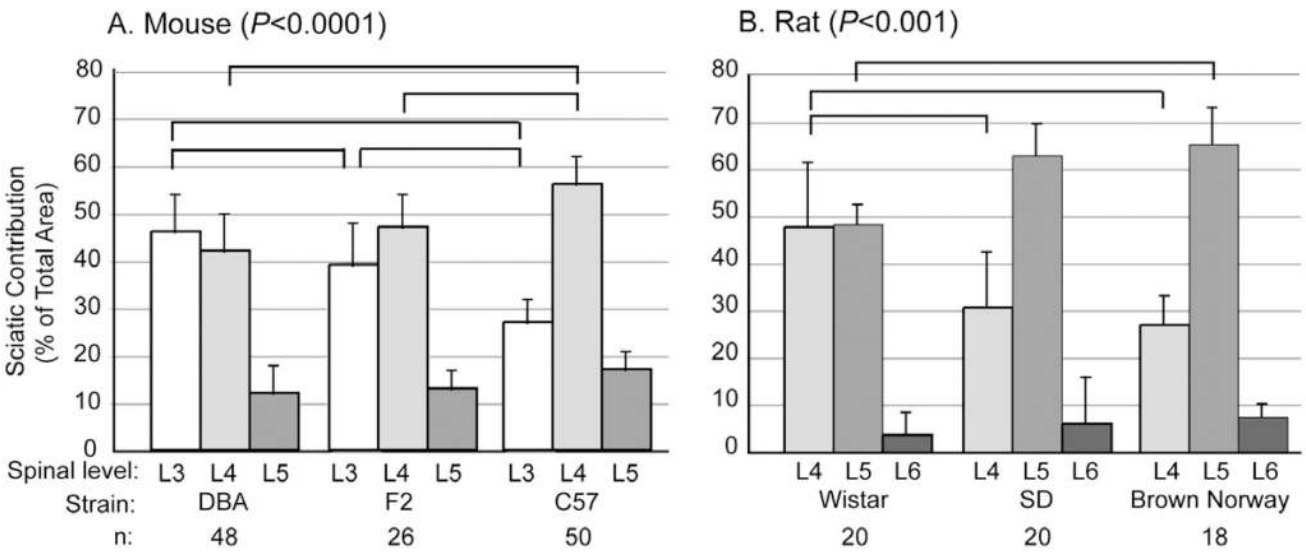

Fig. 3.

Proportionate contributions of different lumbar spinal nerves to the sciatic nerves of mice (A) and rats (B) of different strains. DBA = DBA/2J strain, F2 = B6129PF2/J, C57 = C57BL/6J, SD = Sprague–Dawley. The sciatic nerve is composed of components from the third lumbar (L3) through L5 levels in mice and the L4 through L6 levels in rats. In all strains, the most caudal segment contributes the least. There are significant differences between strains (shown by brackets) in the contribution of each level. The probability of the main effect for the interaction of strain and segmental level on cross-sectional area is shown in parentheses.

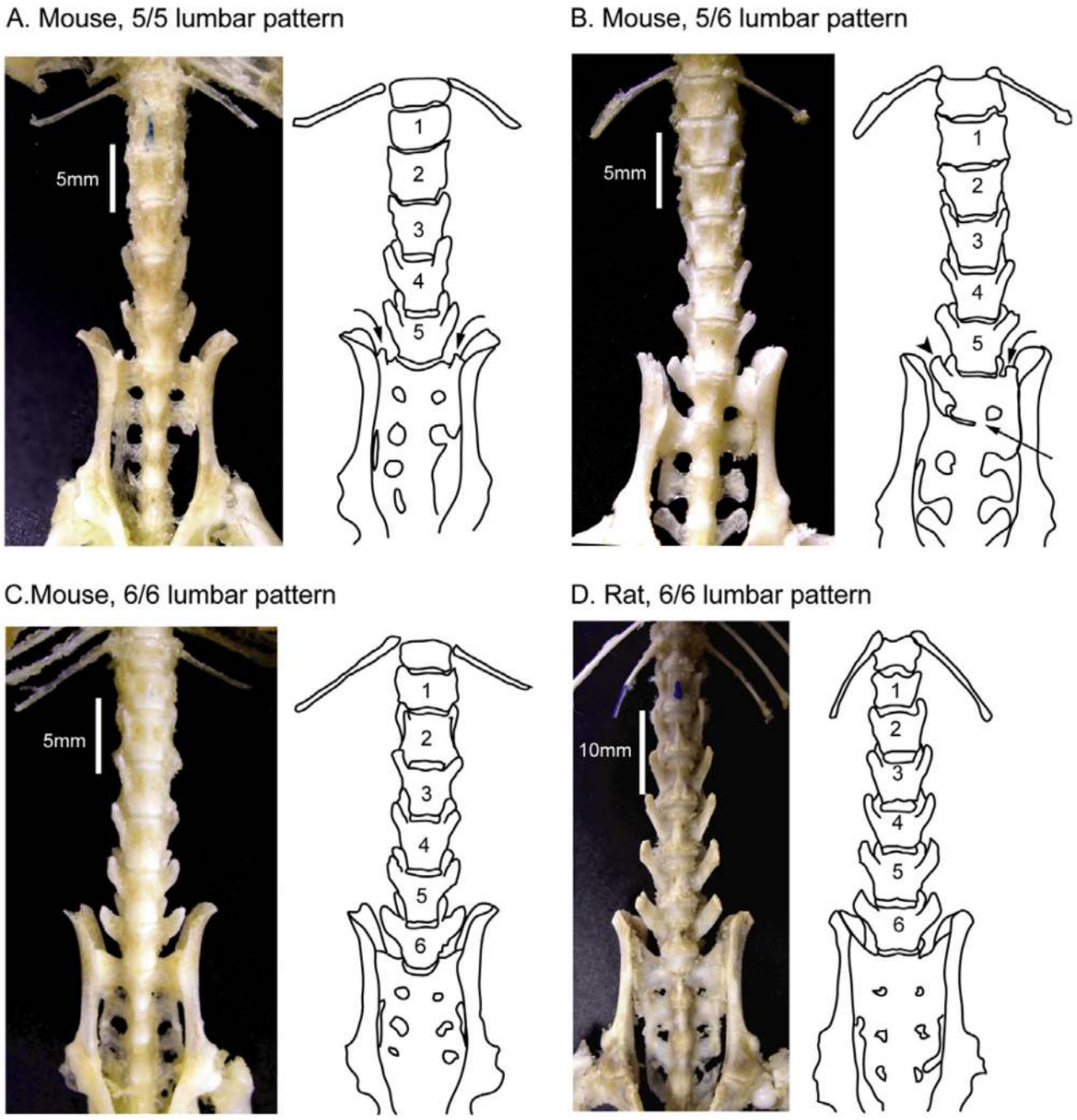

Fig. 4.

Skeletal preparations of mouse and rat vertebral columns and pelvis viewed from the ventral aspect, with reference line drawings. (A) B6129PF2/J strain mouse with five distinct lumbar vertebral segments bilaterally (5/5, i.e. five segments on the left and five on the right). Curved arrows indicate vestigal transverse processes of the fused sixth infra-thoracic vertebra, which is in this case the first sacral (S1) vertebra. (B) C57BL/6J strain mouse with an asymmetric fusion of the sixth lumbar (L6) vertebra (5/6 configuration). On the anatomic left, the L6 transverse process is fused to the ilium and to the sacral ala, leaving a vestigal transverse process (curved arrow), in contrast to the free L6 transverse process on the right (arrowhead). The left side of the L6 vertebral body is fused with the S1 vertebral body (straight arrow). (C)

B6129PF2/J strain mouse with five distinct lumbar vertebral segments bilaterally (6/6 configuration). (D) Sprague–Dawley rat with 6/6 lumbar vertebral configuration.

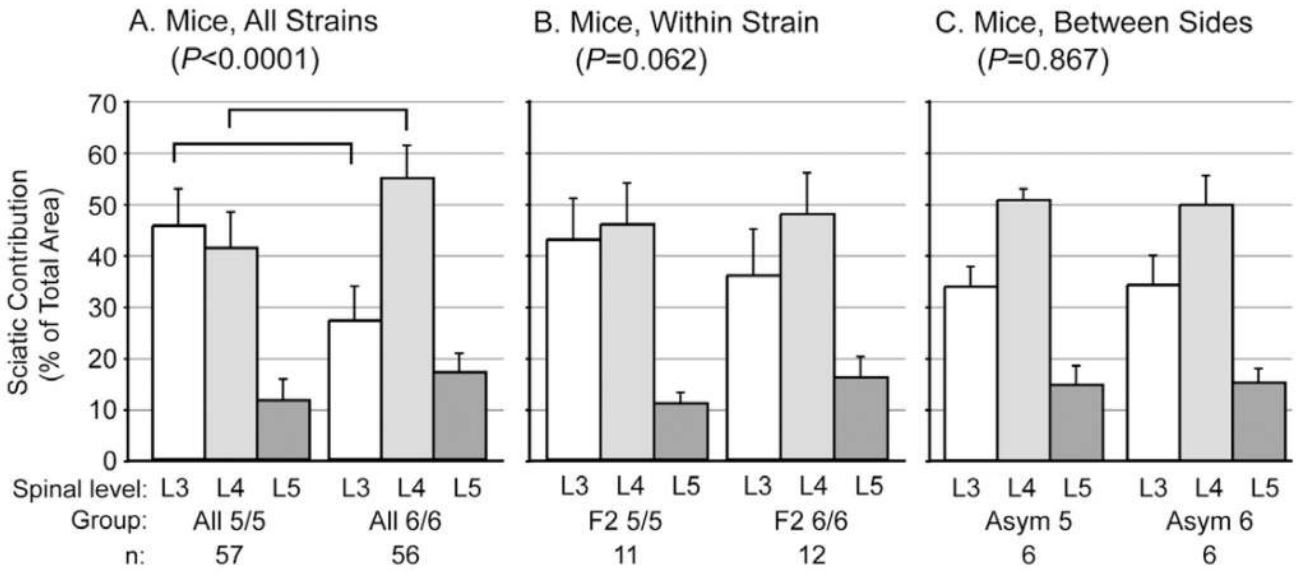

Fig. 5.

Influence of bony segmentation pattern on proportionate contributions of different lumbar spinal nerves to the sciatic nerves of mice. (A) Animals from all three strains (DBA/2J, B6129PF2/J, C57BL/6J) were grouped to compare those with five lumbar vertebrae on each side (5/5) to those with six on each side (6/6). (B) Animals of the B6129PF2/J (F2) strain with 5/5 a pattern were compared to those with a 6/6 pattern. (C) For animals with asymmetric fusion of all strains, the fused side (Asym five) was compared to the non-fused side (Asym six). The probability of the main effect for the interaction of strain and segmental level on cross-sectional area is shown in parentheses. The *n* for each group refers to number of sides. Significant differences between groups at a given level are shown by brackets.

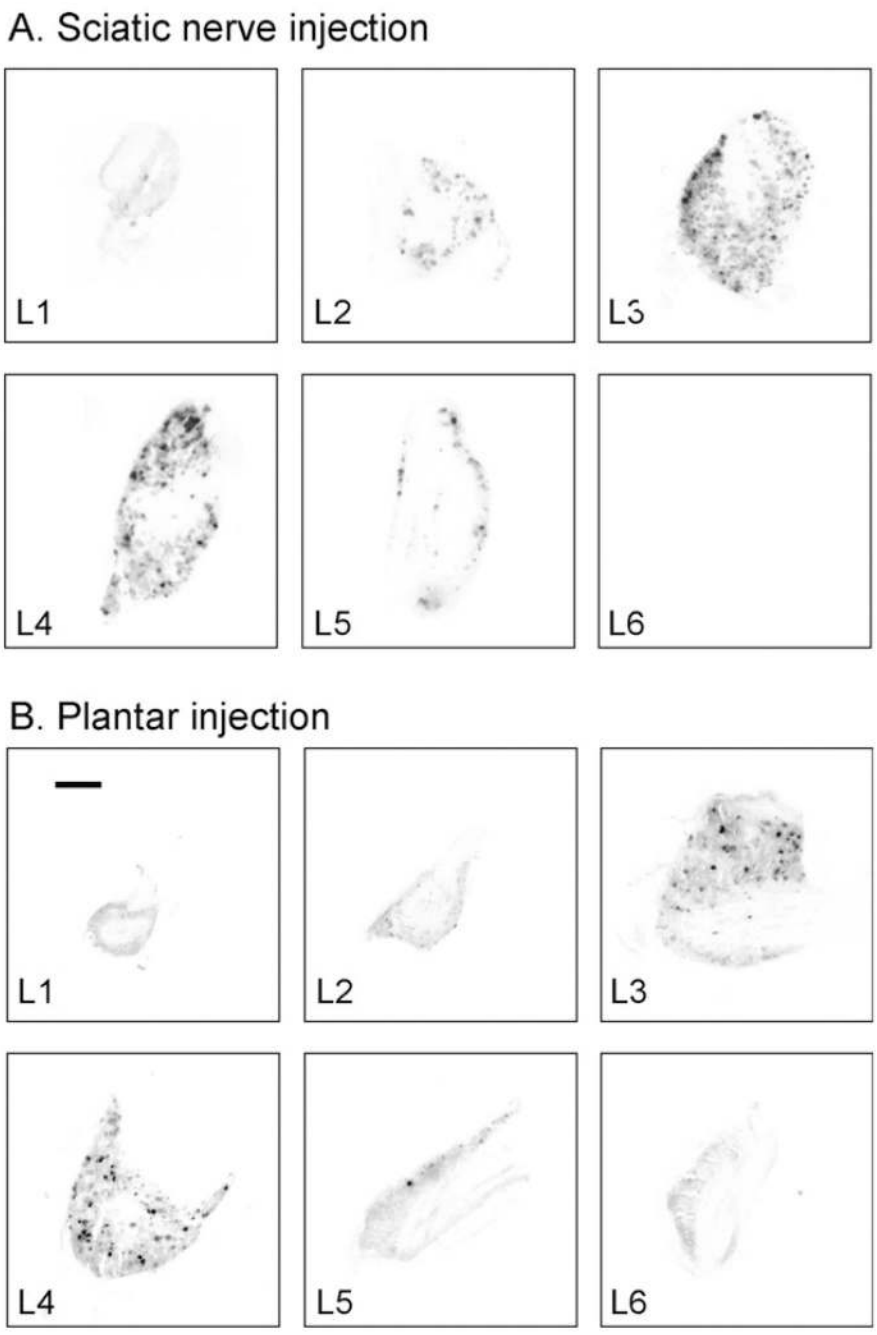

Fig. 6.

Fluorescence photomicrographs (inverted) of representative sections from the first lumbar (L1) through L6 dorsal root ganglia of a C57BL/6J strain mice after injection of True Blue retrograde tracer into (A) the sciatic nerve in the thigh and (B) the plantar aspect of the hind paw of two different animals. In these and all other injected mice, positive sensory neuron somata were found predominantly in the L3 and L4 ganglia, with a smaller population in the L5 ganglion. Counts were made after thresholding the image, which was not done for the images shown here. Scale bar indicates 250 μm and applies to all frames.

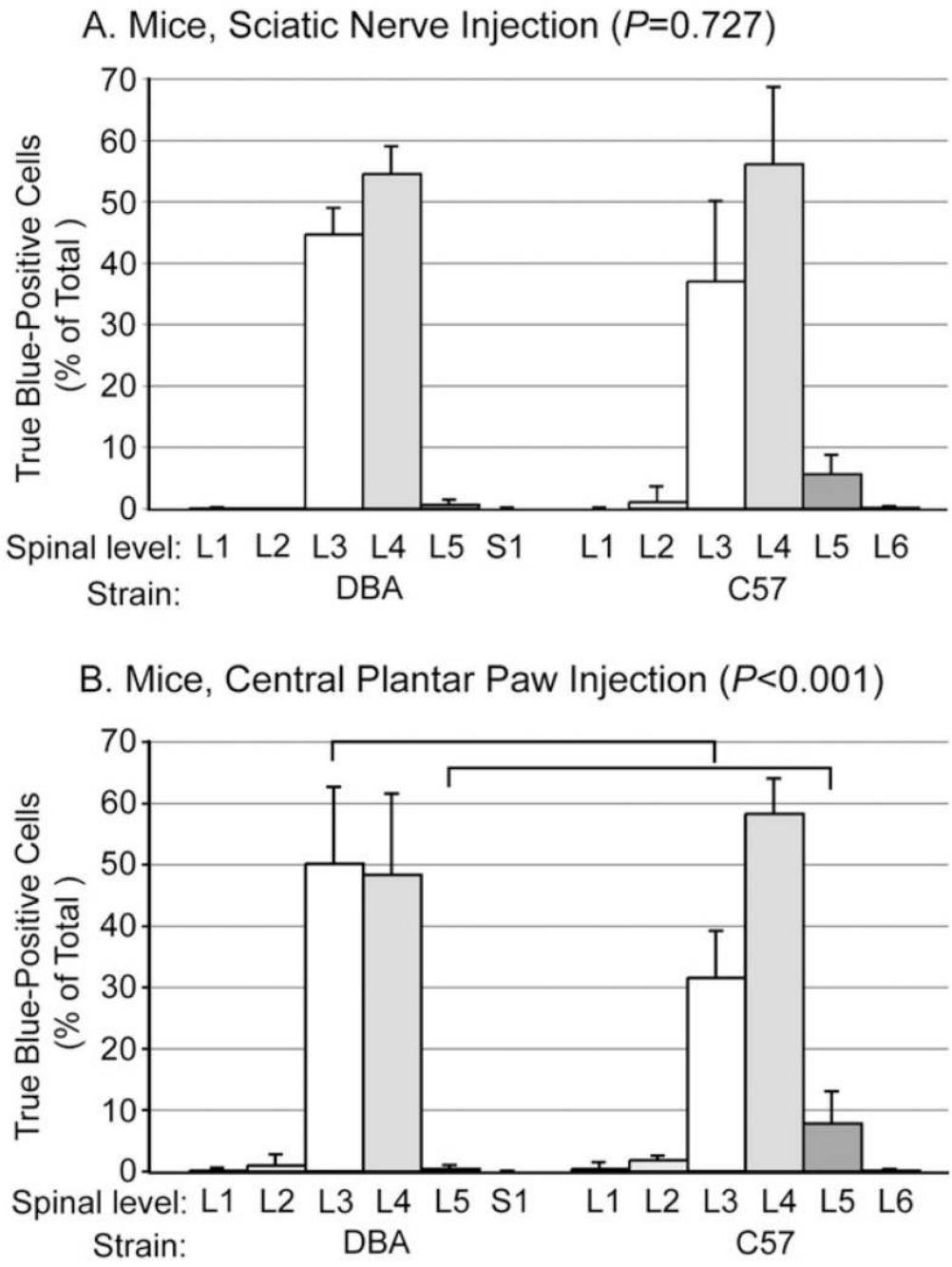

Fig. 7.

Segmental distribution of somata of sensory neurons that project to the thigh (A) or the plantar aspect of the hind paw (B), determined by injection of True Blue tracer at these sites. Data are compared between two strains. The animals of the DBA/2J group (DBA; $n = 5$ for each site) all had five symmetric lumbar vertebrae, while the animals of the C57BL/6J group (C57; $n = 5$ for each site) all had six symmetric lumbar vertebrae. The patterns of contributions determined this way duplicate the findings of nerve dimensions (Fig. 3). The probability of the main effect for the interaction of strain and segmental level on cell counts is shown in parentheses. Significant differences between groups at a given level are shown by brackets.

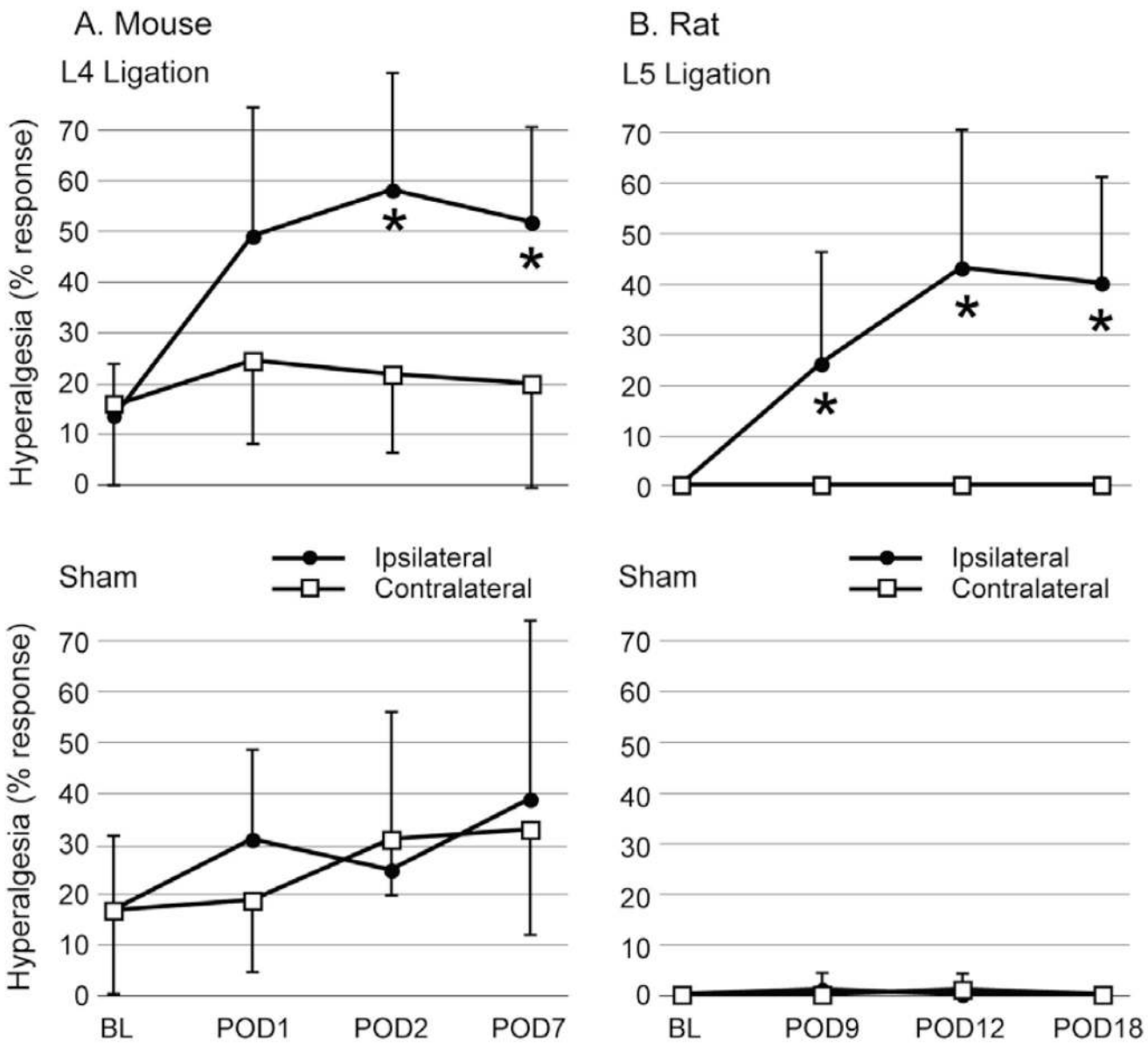

Fig. 8.

Frequency of hyperalgesia responses (sustained lifting, grooming, shaking, or stomping of the foot) in response to nociceptive mechanical stimulation of the plantar aspect of the hind foot with a pin, in mice (A) and in rats (B). The top panels show the response rate ipsilateral and contralateral to spinal nerve ligation (means ± SD), specifically of the L4 in the mice ($n = 11$ ANOVA main effect for side, comparing ipsilateral and contralateral, $P < 0.001$) and L5 in the rats ($n = 10$, $P < 0.001$). Positive post hoc comparisons by Bonferroni test are indicated by *. The bottom panels show the response in sham-operated animals (mice $n = 5$; rats $n = 10$), which resulted in no difference in response between ipsilateral and contralateral sides.

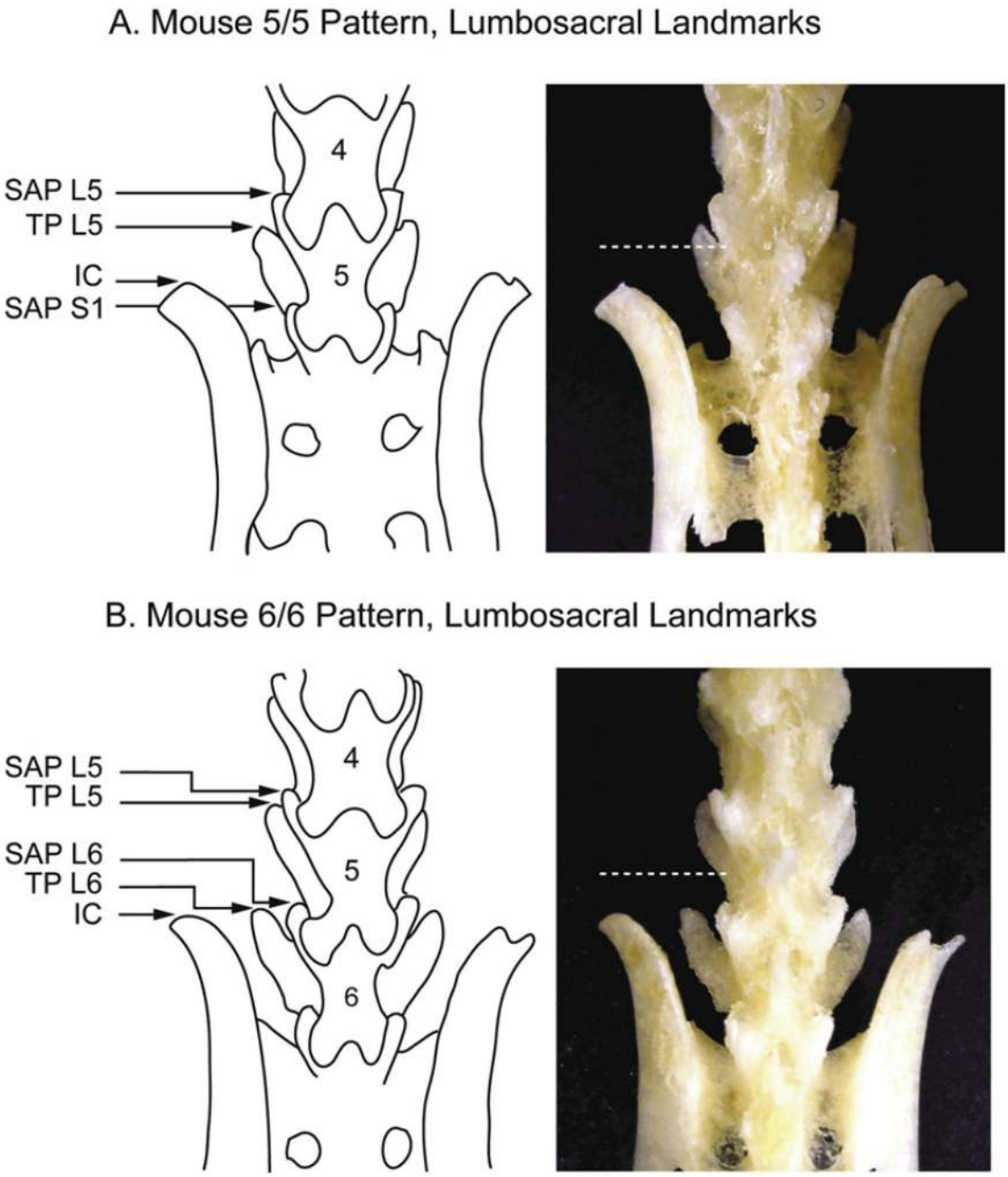

Fig. 9.

Lumbosacral vertebral column of B6129PF2/J strain mice from the posterior aspect, demonstrating sites of measurement for bony landmarks, with references line drawings. (A) A subject with five symmetric lumbar vertebrae, showing iliac crest (IC), transverse process (TP) of the fifth lumbar vertebra (L5), and the superior articular processes (SAP) of the first sacral (S1) and L5 vertebrae. (B) Similar measurements in a subject with six symmetric lumbar vertebrae. In both images, the dotted line indicates a level 1 mm above the IC. The L5 SAP is always more than 1 mm rostral to the IC, shown in these two animals.

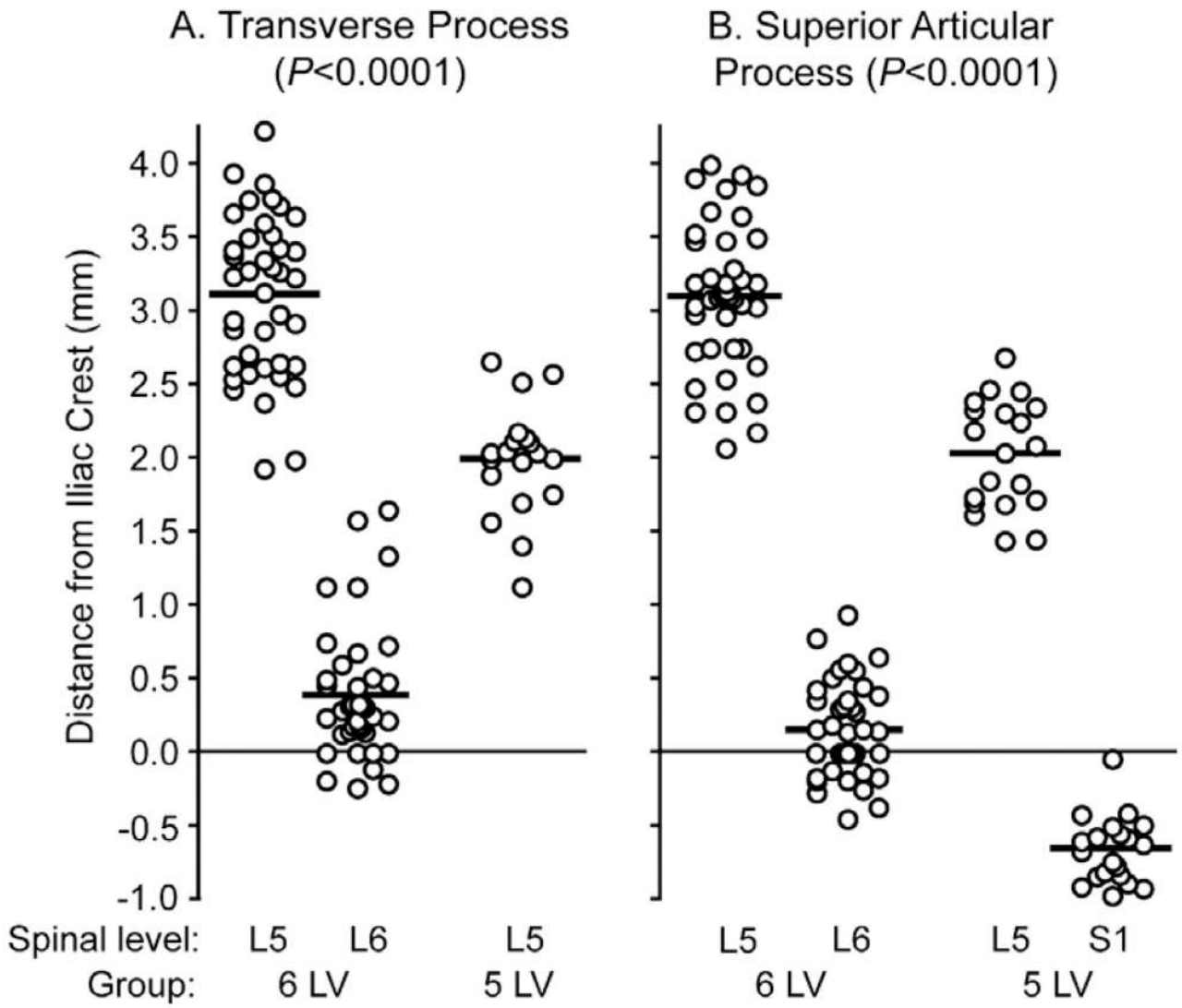

Fig. 10.

Location of anatomic landmarks relative to the superior edge of the iliac crest. (A) The distance from the iliac crest to the rostral edge of the ipsilateral transverse process (A) was determined for the fifth lumbar (L5) and L6 processes in animals that have six lumbar vertebrae (six LV) and for the L5 of animals with five lumbar vertebrae (five LV). Positive dimensions indicate a rostral direction from the iliac crest. All groups were significantly different. (B) Similar measurements were made for the distances from the iliac crest to the rostral edge of the L5 and L6 superior articular processes of animals with six lumbar vertebrae and the L5 and first sacral (S1) superior articular processes of animals with five lumbar vertebrae. All groups were significantly different. Bars indicate means.

Table 1

Frequency of neural connections between adjacent spinal levels in the lumbosacral plexus of various mouse and rat strains

Levels	Connect	Strain		
		DBA	**F2**	**C57**
Mice				
L2 and L3	Yes	29 (51)	35 (90)	52 (100)
(P <0.001)	No	28 (49)	4 (10)	0 (0)
L3 and L4	Yes	60 (100)	40 (100)	52 (100)
(no variance)	No	0 (0)	0 (0)	0 (0)
L4 and L5	Yes	52 (93)	38 (95)	51 (98)
(P = 0.44)	No	4 (7)	2 (5)	1 (2)
		Wistar	SD	BN
Rats				
L3 and L4	Yes	13 (81)	10 (53)	16 (100)
(p = 0.004)	No	3 (19)	9 (47)	0 (0)
L4 and L5	Yes	20 (100)	20 (100)	18 (100)
(no variance)	No	0 (0)	0 (0)	0 (0)
L5 and L6	Yes	8 (50)	14 (70)	18 (100)
(p = 0.004)	No	8 (50)	6 (30)	0 (0)

Numbers in parentheses are % of total for that strain. Data are not included if the cases where the tissue may have been damaged. DBA = DBA/2J strain, F2 = B6129PF2/J, C57 = C57BL/6J, SD = Sprague–Dawley, BN = brown Norway, "*n*" indicates the number of sides. *P* indicates the significance level of the influence of strain on inter-segment connections.

Table 2

Number of lumbar vertebral bony segments in various mouse and rat strains

Segments, left/right	**Strain**		
	DBA, *n* = 29	**F2, *n* = 20**	**C57, *n* = 37**
Mice (P < 0.001)			
5/5	28 (97)	6 (30)	2 (6)
5/6, 6/5	1 (3)	2 (10)	4 (8)
6/6	0	12 (60)	31 (86)
	Wistar, *n* = 10	SD, *n* = 16	BN, *n* = 9
Rats (no variance)			
5/5	0	0	0
5/6, 6/5	0	0	0
6/6	10 (100)	16 (100)	9 (100)

Numbers in parentheses are % of total for that strain. The number of free lumbar vertebrae is specified left/right. Asymmetric fusion is specified by 5/6, (the transverse process of the last lumbar vertebra is fused to the sacrum and articulates with the ilium on the left but free on the right) and 6/5 (the reverse pattern). DBA = DBA/2J strain, F2 = B6129PF2/J, C57 = C57BL/6J, SD = Sprague–Dawley, BN = brown Norway. "*n*" indicates the number of animals. *P* indicates the significance level of the influence of strain on segmentation pattern.