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Research report

# Effect of *Brn-3a* deficiency on parvalbumin-immunoreactive primary sensory neurons in the dorsal root ganglion

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**Abstract**

Immunohistochemistry for parvalbumin, a marker for primary proprioceptors, was performed on the dorsal root ganglion (DRG) of wildtype and knockout mice for *Brn-3a* at postnatal day 0 and embryonic day 18.5. The DRG contained many parvalbumin-immunoreactive (ir) neurons in wildtype (5.4%) and knockout mice (5.6%). Cell size analysis demonstrated that such neurons were mostly medium-sized to large in these mice. Therefore, it is unlikely that the survival of proprioceptors is dependent upon *Brn-3a* in the DRG. In the dorsal column and gray matter of the spinal cord of knockout mice, however, parvalbumin-ir nerve fibers were sparse compared to wildtype mice. The number of parvalbumin-ir varicosities around motoneurons decreased in the mutant. Thus, our data suggest that *Brn-3a* may play an important role in the central projection and terminal formation of DRG proprioceptors in the spinal cord.


*Theme:* Development and regeneration
*Topic:* Sensory systems

*Keywords:* Transcription factor; Knockout mouse; Parvalbumin; Proprioceptor; Immunohistochemistry

*Brn-3a* is a member of the POU family of transcription factors which is predominantly expressed by neurons [3,4]. This factor can induce expression of the *Bcl-2* gene and protect neurons from apoptosis [2,9,10,13]. *Brn-3a* can also activate a number of other neuronally expressed genes and stimulate neuronal process outgrowth [11,12]. During development, the trigeminal and dorsal root ganglia (DRG) show intense *Brn-3a* expression [14,15]. A previous study demonstrated that targeted deletion of the *Brn-3a* gene results in a marked reduction of neurons in the trigeminal ganglion [5,15]. Such extra cell death could not be detected in the DRG. Therefore, the survival of primary sensory neurons is probably dependent upon *Brn-3a* in the trigeminal ganglion but not in the DRG. However, *Brn-3a* deficiency may have some effect on muscular proprioceptors in the DRG because the knockout mouse shows uncoordinated limb movement [15].

Parvalbumin is a member of calcium-binding protein family. Previous immunohistochemical studies have demonstrated that this protein is localized to large neuronal cell bodies in the DRG [1,6]. These neurons innervate the musculature, and emit their terminals to the muscle spindle [1]. Therefore, parvalbumin-ir neurons are considered to be muscular proprioceptors in the DRG. *Brn-3a* deficiency may have an effect on the survival and projection of parvalbumin-ir neurons in the DRG. However, the distribution of parvalbumin-immunoreactivity (ir) has never been reported in the DRG, muscle or spinal cord of the knockout mouse.

In this study, the distribution of parvalbumin-ir DRG neurons and their central and peripheral projections were examined in wildtype and *Brn-3a* knockout mice.

Mice lacking expression of *Brn-3a* were prepared according to the method of Xiang et al. [15]. Two wildtype

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and four knockout mice at embryonic day 18.5 (E18.5), and five wild and six knockout mice at postnatal day 0 (P0) were obtained from mating of the heterozygous mice. Animals were killed by decapitation, and immersed overnight in the fixative at 4 °C. Subsequently, tissues were stored at 4 °C in phosphate-buffered saline (PBS) containing 0.1 mM sodium azide until used. The forelimbs and hind limbs, and the vertebral column including the DRGs and spinal cords were dissected, immersed in PBS containing 20% sucrose overnight, frozen-sectioned at 10 μm, and thaw-mounted on gelatin-coated glass slides. These sections were incubated with rabbit anti-parvalbumin serum (1:50,000, Swant, Switzerland) followed by the incubation with biotinylated goat anti-rabbit IgG and avidin–biotin–horseradish peroxidase complex (Vector Laboratories). For cell size analysis of parvalbumin-stained neurons, microscopic images ( × 215) of cell bodies were projected over a digitizer tablet using a drawing tube. The cross-sectional area of those cell bodies that contained the nucleolus was recorded.

For the image analysis of the spinal cord at P0, the optic image of each section was captured with a digital camera

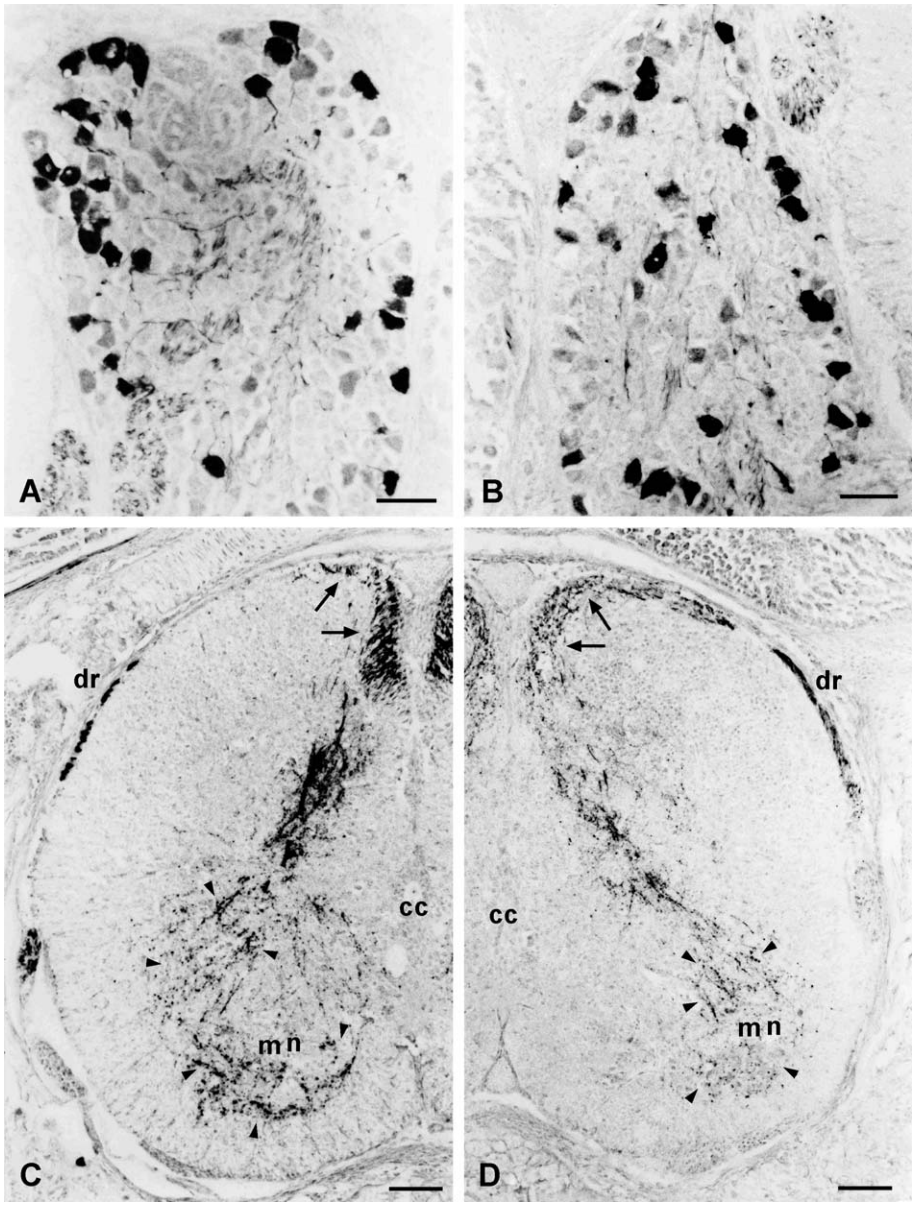


Fig. 1. Immunohistochemical microphotographs of parvalbumin-ir in the DRG (A, B) and spinal cord (C, D) of wildtype (A, C) and knockout (B, D) mice at P0 (A, B) and E18.5 (C, D). The DRG contained many parvalbumin-ir neurons in wildtype (A) and knockout (B) mice. In the spinal cord of the wildtype mouse, parvalbumin-ir fibers are detected in the dorsal column (arrows in C). From the base of the dorsal horn, parvalbumin-ir axons run ventrolaterally toward the ventral horn, which contains many ir varicosities (arrowheads in C). In the knockout mouse, the number of parvalbumin-ir fibers greatly decreases in the dorsal column (arrows in D) and the gray matter (arrowheads in D). In C and D, dr and mn indicate the dorsal root and the motoneuron area, respectively. Bars = 50 μm (A, B) and 100 μm (C, D).

(COOLPIX4500, Nikon, Tokyo, Japan) and stored into a computer (Power Macintosh G4, Apple, Cupertino, USA). The NIH Image program was used to measure the optical density of the spinal cord and background (immunonegative area) in each section. The density of the spinal cord in P0 wildtype and knockout mice was divided by the background density. The quotient was recorded for each spinal cord and will be referred to as the ir density, hereafter. The data for the ir density were obtained from 24 sections from six wildtype mice and five knockout mice, and the difference was statistically analyzed with a *t*-test.

The specificity of the primary antisera used in this study has been described elsewhere [6].

The experiments were carried out under the control of the Animal Research Control Committee in accordance with The Guidelines for Animal Experiments of Okayama University Medical School, Government Animal Protection and Management Law (No. 105), and Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6). All efforts were made to minimize the number of animals used and their suffering.

The DRG contained many parvalbumin-ir neurons in wildtype (Fig. 1A) and knockout (Fig. 1B) mice. The proportion of parvalbumin-ir neurons was similar in the DRG of these mice; 5.4% (124/2311) and 5.6% (115/2073) of DRG neurons in wildtype and mutant mice, respectively, were immunoreactive for parvalbumin. Cell size analysis demonstrated that parvalbumin-ir neurons were mostly medium-sized to large in P0 wildtype (mean ± S.D. = 321.0 ±98.2 $\mu m^2$, range = 137.4–594.8 $\mu m^2$, $n = 170$) and knockout mice (mean ± S.D. = 299.6 ± 95.1 $\mu m^2$, range = 76.0–659.5 $\mu m^2$, $n = 137$). About half of parvalbumin-ir neurons were larger than 300 $\mu m^2$ in these mice (52.9% or 90/170 for wildtype mice, and 48.2% or 66/137 for knockout mice). More than 30% of parvalbumin-ir neurons measured 200–300 $\mu m^2$ (35.3% or 60/170 for wildtype mice, and 38.0% or 52/137 for knockout mice). A small proportion of parvalbumin-ir neurons had cell bodies $< 200$ $\mu m^2$ (11.8% or 20/170 for wildtye mice, and 13.9% or 19/137 for knockout mice).

The spinal cord contained parvalbumin-ir fibers but not neuronal cell bodies in wildtype (Fig. 1C) and knockout (Fig. 1D) mice. In wildtype mice at E18.5 and P0, parvalbumin-ir fibers were detected in the dorsal column (Fig. 1C). The head of the dorsal horn was almost devoid of the ir fibers but the base of the dorsal horn contained many ir fibers. After giving off varicose-bearing terminal arbors in the intermediate gray matter, these axons appeared to continue ventrolaterally toward the ventral horn (Fig. 1C). In the ventral horn, numerous ir varicosities were seen surrounding immunonegative motoneurons. In E18.5 and P0 knockout mice, however, the number of parvalbumin-ir fibers decreased dramatically compared to wildtype mice. Parvalbumin-ir fibers in the dorsal column and the gray matter were equally affected. The difference of the mean density of parvalbumin-ir between the wildtype (mean ± S.E. = 1.067 ± 0.003, range = 1.04–1.125, $n = 24$) and knockout mice (mean ± S.E. = 1.040 ± 0.004, range = 1.027–1.077, $n = 24$) for each spinal cord was highly significant ($P < 10^{-5}$, a *t*-test).

In contrast, the relatively strong ir did not seem to have decreased in the dorsal root (Fig. 1D).

In forelimbs and hind limbs of P0 wildtype mice, we found that thick and thin nerve bundles contained numerous parvalbumin-ir nerve fibers. They entered the musculature

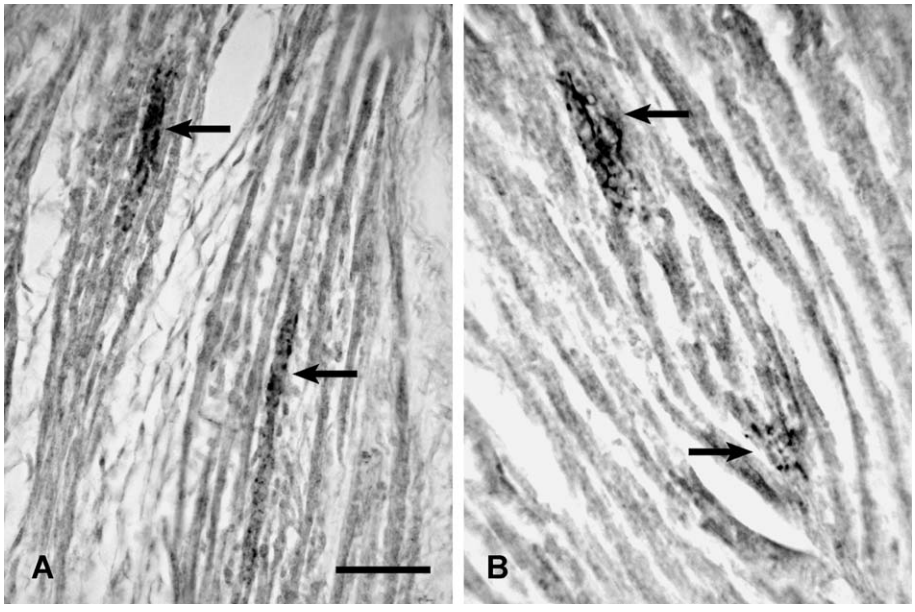


Fig. 2. Immunohistochemical microphotographs of parvalbumin-ir in the skeletal muscle of the hind limb. The muscle spindles contained parvalbumin-ir nerve terminals (arrows) in wildtype (A) and knockout (B) mice. The distribution of muscle spindles and parvalbumin-ir terminals was similar in these mice. Bar = 50 μm (A). Panels A and B are at the same magnification.

and ran toward many muscle spindles. In the muscle spindle, parvalbumin-ir nerve fibers formed spiral and varicose axon terminals closely apposed to intrafusal muscle fibers (Fig. 2A). We observed a similar distribution of muscle spindles and parvalbumin-ir nerve fibers in knockout mice (Fig. 2A,B).

The present study investigated the distribution of parvalbumin-ir neurons in the DRG of wildtype and knockout mice for *Brn-3a*. The DRG contained many parvalbumin-ir neurons in these mice. In the spinal cord, parvalbumin-ir nerve fibers were detected in the dorsal column and the gray matter. These nerve fibers are considered to originate from the DRG, because parvalbumin-ir cell bodies could not be observed in the spinal cord.

In our previous study, we found that the number of parvalbumin-ir neurons decreased in the trigeminal ganglion of *Brn-3a* knockout mice [7]. The loss of *Brn-3a* caused a significant decrease of parvalbumin-ir nerve fibers in the infraorbital nerve and vibrissa. Therefore, the survival of parvalbumin-ir low-threshold mechanoreceptors is thought to depend on this transcription factor. In the present study, however, we could show that the proportion and size spectrum of parvalbumin-ir DRG neurons were similar in wildtype and knockout mice. Because the loss of *Brn-3a* has no effect on the number of DRG neurons [15], it is unlikely that *Brn-3a* deficiency affects the survival of parvalbumin-ir neurons or the expression of parvalbumin in the DRG. This is in contrast to our previous observation about calcitonin-gene-related peptide (CGRP)-ir DRG neurons in *Brn-3a* knockout mice [8]. Medium-sized and large CGRP-ir neurons greatly decreased in the knockout mice. On the other hand, the number of small CGRP-ir DRG neurons concomitantly increased in the mutants. The loss of *Brn-3a* probably caused the increase and decrease of CGRP-ir expression in small and large DRG neurons, respectively. *Brn-3a* may control the expression of CGRP but not parvalbumin in DRG neurons.

In forelimbs and hind limbs of both wildtype and knockout mice, skeletal muscles contained many muscle spindles. Within the spindle, nerve terminals were immunoreactive for parvalbumin. By contrast, parvalbumin-ir fibers were sparse in the spinal cord of knockout mice compared to wildtye mice. The ir density in the spinal cord of wildype mice was statistically higher than in that of knockout mice. The number of parvalbumin-ir varicosities around motoneurons markedly decreased in the mutant mouse. Therefore, *Brn-3a* deficiency appears to have an effect on the distribution of DRG-derived parvalbumin-ir in the spinal cord but not in the peripheral tissues, suggesting that *Brn-3a* may be involved in the central projection and terminal formation of DRG proprioceptors in the spinal cord. DRG neurons are considered to require trophic support from their central projections for survival. If this was the case, the survival of parvalbumin-ir neurons in *Brn-3a* knockout mice may be impaired by loss of their central projections during postnatal development.

In conclusion, the present study has described parvalbumin-ir DRG neurons in *Brn-3a* knockout mice. It is unlikely that the loss of *Brn-3a* has an effect on the survival or peripheral projection of parvalbumin-ir DRG neurons. However, *Brn-3a* may be involved in the central projection of parvalbumin-ir DRG neurons and their terminal formation in the spinal cord.

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