Research and Report

GDF8 and GDF11 expression in the adult rat trigeminal nuclei

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Abstract

Growth and differentiation factor 8 (GDF8, also called as myostatin) and GDF11 (also known as bone morphogenetic protein 11, BMP11) are members of the transforming growth factor β (TGF β) superfamily. Although the deep involvement of GDF signaling in the development and functions of the trigeminal nuclei has been postulated, little information is available for its expression in the trigeminal nuclei. We, thus, investigated GDF8 and GDF11 expression in the adult rat trigeminal nuclei. Frozen brains of Wistar rats (n=8, 7 weeks old) were cut on a cryostat and immunohistochemistry was performed using specific antibodies against GDF8 and GDF11. GDF8 and GDF11 were intensely expressed throughout the trigeminal nuclei. They were expressed in nuclei, cytoplasm and neuropil. In addition, we also detected GDF8 and

GDF11 expression in axons of the trigeminal nerve. These data indicate that GDF8 and GDF11 play pivotal roles also in the adult trigeminal system.

Keywords: GDF8, GDF11, axon, immunohistochemistry

Introduction

Growth and differentiation factor 8 (GDF8) (also called as myostatin) and GDF11 (also known as bone morphogenetic protein 11; BMP11), are members of the transforming growth factor β (TGF β) superfamily [1,2]. GDF 8 and 11 are synthesized as precursor molecules, and after dimerization, the N-terminal prodomains are cleaved from the C-terminal signaling domains by a furin protease, and the prodomains are further cut by a tolloid-like metalloproteinase, finally making the active ligands [2]. GDF 8 and 11 use the type II receptors activin receptor kinase IIA and IIB and the type I receptors activin receptor-like kinase 4 (ALK4), ALK5 [2]. On GDF8 and 11 binding, the type I receptors activate the receptor activated Smads (R-Smads; Smad2/3) which bind with common-mediator Smad (Co-Smad; Smad4). The Smad complex then moves to the nucleus and plays as a transcription regulator [2].

Gene mutations and deletions for GDF8 cause hypertrophic growth of skeletal muscles in mice [3] and in humans [4]. GDF8 has been also reported to be involved in cardiac growth and metabolism [2], postnatal glucose metabolism and adipogenesis [5]. In the central nervous system (CNS), GDF8 is also known to reduce neurite outgrowth and inhibitory synapse formation [6], and increase the number of surviving retinal ganglion cells with neurite extension [7]. GDF11 also plays pivotal roles during development, including anterior/posterior patterning, formation of the kidney, stomach, spleen and endocrine pancreas [8-11]. In the CNS, GDF11 is reported to facilitate temporal progression of neurogenesis [12], and to control the numbers of retinal ganglion cells, as well as amacrine and photoreceptor cells during the development of the retina [11]. Above-mentioned data strongly indicate that GDF8 and 11 play pivotal roles in many biological events.

Also in the trigeminal system, Hodge et al. have shown that retrograde BMP4 signaling regulates trigeminal sensory neuron identities and the formation of precise face maps [13]. In addition, Strelau et al. have reported that GDF15-deficient mice exhibit progressive postnatal losses of trigeminal motor

neurons [14]. These data strongly suggest that GDF signaling are deeply involved in the development and functions of trigeminal system. However, little information is available for GDF8 and 11 expression in the adult trigeminal nuclei. It is, thus, necessary to perform detailed investigations of the expression pattern of GDF8 and 11 in the adult trigeminal nuclei.

Materials and methods

1. Animals and section preparation

Under deep anesthesia, brain samples were isolated from male Wistar rats (n = 8, 7 weeks old; Japan SLC Inc., Shizuoka Japan). For immunohistochemistry, the rats were perfused transcardially with saline followed by 0.1 M phosphate buffer (PB, pH 7.4) containing 4% paraformaldehyde and 0.2% picric acid. The brains were removed rapidly, and then postfixed in the same fixative for 2 hr at 4 °C. All brains were immersed in 10%, 20%, 25% buffered sucrose for overnight at 4 °C respectively. Frozen sections (20 m in thickness) were cut on a cryostat. All animal experiments in this study conformed to the Guidelines for Animal Experiments at Hamamatsu University School of Medicine on the ethical use of animals.

2. Immunohistochemistry

For immunoperoxidase staining, the sections were treated with 10 % normal goat serum and 0.2 % Triton X-100 in 0.1 M PB for 2 hr at room temperature, and incubated further with primary antibodies overnight at 4 °C. After being washed with 0.1 M PB, sections were incubated with secondary antibodies for 2 hr at room temperature. After being washed with 0.1 M PB, immunoreaction was visualized with 3,3'-diaminobenzidine (Wako, Osaka, Japan).

A rabbit anti-GDF8 antibody (diluted 1:200, ab71808; Abcam plc, Cambridge, UK) and a mouse anti-GDF11 antibody (diluted 1:100, the final concentration; R&D Systems, Inc., Minneapolis, MN) were used as primary antibodies. And a goat anti-rabbit IgG with peroxidase complex (no dilution, EnVision+ System-HRP; DAKO, Glostrup, Denmark) and a goat anti-mouse IgG with peroxidase complex (no dilution; Histofine Simple Stain Rat MAX-PO(M); NICHIREI BIOSCIENCE INC., Tokyo, Japan) were used as secondary antibodies.

Bright-field images were obtained using a microscope (Eclipse 80i; Nikon, Tokyo, Japan). They were further

processed in image analysis software (Photoshop; Adobe, Tokyo, Japan).

Results

General expression patterns

The specificity for the antibodies used in this study has been evaluated in our previous studies [15,16]. Fig. 1 shows the overview of GDF8 and GDF11 expression in the adult trigeminal nuclei. GDF8-like immunoreactivity (IR) and GDF11-IR were observed throughout the trigeminal nuclei. Both proteins were abundantly expressed in the mesencephalic trigeminal nucleus, motor trigeminal nucleus, principal trigeminal nucleus (Fig. 1A and B), oral part of the spinal trigeminal nucleus (Fig. 1C and D), and caudal part of the spinal trigeminal nucleus (Fig. 1E and F). The relative intensity of chordin-IR and noggin-IR in the rat trigeminal nuclei is summarized in Table 1.

Mesencephalic trigeminal nucleus

GDF8 and GDF11-IRs were abundantly seen in the mesencephalic trigeminal nucleus (Fig. 2A-F). In the mesencephalic trigeminal nucleus, the majority of the neurons are large primary afferent neurons, which transmit information from muscle spindles of jaw muscles and periodontal receptors of both maxillary and mandibular teeth [17]. Interestingly, GDF8-IR was abundantly observed in the nuclei of large primary afferent neurons in different levels (Fig. 2C and D). Some neurons expressed it very strongly (arrowheads in Fig. 2D) and the others moderately (arrows in Fig. 2D). In addition, GDF8-IR was also weakly observed in cytoplasm of neurons and neuropil. In contrast, GDF11-IR was strongly seen in both nuclei and cytoplasm (arrowheads in Fig. 2F). Furthermore, moderate GDF11-IR was observed in neuropil (Fig. 2E and F).

Motor trigeminal nucleus

GDF8 and GDF11-IRs were also abundantly observed in the motor trigeminal nucleus (Fig. 2G-L). The motor trigeminal nucleus contains large motor neurons innervating jaw closing muscles [18]. Interestingly, GDF8-IR was very strongly observed in the nuclei of large motor neurons (arrowheads in Fig. 2J). In addition, GDF8-IR was also observed moderately in cytoplasm of neurons, and weakly in neuropil (Fig. 2I and J). In contrast, GDF11-IR was strongly seen in both nuclei and cytoplasm (arrowheads in

Fig. 2L). Furthermore, weak GDF11-IR was observed in neuropil (Fig. 2K and L).

Principal trigeminal nucleus

Abundant GDF8 and GDF11-IRs were also seen in the principal trigeminal nucleus (Fig. 3A-F). This nucleus contains a high density of medium and small neurons [17]. Interestingly, GDF8-IR was very strongly observed in the nuclei of many neurons (arrowheads in Fig. 3D). In addition, GDF8-IR was also observed moderately in cytoplasm of neurons, and weakly in neuropil (Fig. 3C and D). In contrast, GDF11-IR was strongly detected in both nuclei and cytoplasm (arrowheads in Fig. 3F). Furthermore, weak GDF11-IR was observed in neuropil (Fig. 3E and F).

Oral part of the spinal trigeminal nucleus

In the oral part, some very large neurons with widespread dendritic arbors as well as medium and small neurons are observed [17]. Abundant GDF8 and GDF11-IRs were also seen in this part (Fig. 3G and H). Interestingly, GDF8-IR was strongly observed in the nuclei of many neurons (arrowheads in Fig. 3J). In addition, GDF8-IR was also observed moderately in cytoplasm of neurons, and weakly in neuropil (Fig. 3I and J). In addition, GDF8-IR was also weakly observed in cytoplasm of neurons and neuropil. In contrast, GDF11-IR was strongly seen in both nuclei and cytoplasm (arrowheads in Fig. 3L). Furthermore, week GDF11-IR was observed in neuropil (Fig. 3K and L).

Caudal part of the spinal trigeminal nucleus

Like the dorsal horn of spinal cord, caudal part of the spinal trigeminal nucleus has a laminar arrangement [17]. The superficial layer consists of a marginal zone and substantia gelatinosa, and large-sized neurons are scattered in the deep layer [17]. Interestingly, strong GDF8-IR was observed in the nuclei of many neurons (arrowheads in Fig. 4D). In addition, GDF8-IR was also observed moderately in cytoplasm of neurons, and weakly in neuropil (Fig. 4C and D). In addition, GDF8-IR was also weakly observed in cytoplasm of neurons and neuropil. In contrast, strong GDF11-IR was seen in both nuclei and cytoplasm (arrowheads in Fig. 4F). Furthermore, moderate GDF11-IR was observed in neuropil (Fig. 4E and F).

Paratrigeminal nucleus

The paratrigeminal nucleus consists of groups of cells embedded in the dorsal part of the spinal tract just rostral to the obex [17]. This nucleus receives inputs from perioral and intraoral regions and the upper gastrointestinal tract via trigeminal and glossopharyngeal afferents and possibly vagal fibers [17]. This nucleus is also considered to be an important integrating area for nocieceptive somatovisceral reflexes involving the upper gastrointestinal tract [17]. Interestingly, GDF8-IR was strongly observed in the nuclei of many neurons (arrowheads in Fig. 4I). In addition, GDF8-IR was also observed moderately in cytoplasm of neurons and neuropil (Fig. 4I). In contrast, GDF11-IR was very strongly seen in both nuclei and cytoplasm (arrowheads in Fig. 4K). Furthermore, moderate GDF11-IR was observed in neuropil (Fig. 4K).

Trigeminal tract

The trigeminal tract consists of axons from trigeminal ganglia (Fig.4I-L). Interestingly, many axons were positive for both GDF8-IR and GDF11-IR (arrowheads in Fig. 4J and L).

Discussion

To date, GDF8 and GDF11 expression in the trigeminal nuclei have not been investigated. In the present study, we first show that GDF8 and GDF11 are widely expressed in all trigeminal nuclei.

GDF8 and GDF11 expression in neurons

We observed abundant GDF8 and GDF11 in most neurons of the trigeminal nuclei. These trigeminal nuclei contain differently-categorized neurons. For example, the mesencephalic trigeminal nucleus has primary afferent neurons, the motor trigeminal nucleus contains motor neurons. And in the other nuclei, most neurons are secondary afferent neurons that transmit information to the thalamus. In addition, all nuclei also possess interneurons. Interestingly, in the present study, we found almost all neurons express GDF8 and GDF11 proteins, indicating that throughout the trigeminal nuclei GDF8 and GDF11 play important roles.

GDF8 and GDF11 expression in cell nuclei

In many neurons, we detected strong GDF8 and GDF11 expression in nuclei.

Interestingly, Artaza et al. have also reported that, in C2C12 skeletal muscle cells, GDF8 is also expressed in nuclei [19]. In addition, the nuclear localization of GDF8 has been also reported in H9c2 embryonic cardiomyocytes [20]. The authors speculate that GDF8 in nuclei may play a role to inhibit muscle proliferation via down-regulation of genes critical for cell proliferation, such as cyclins, and that GDF8 in nuclei may be involved in transcriptional regulation [19]. Since it is well known that mature neurons do not have proliferating ability, these reports and our finding raise the possibility that GDF8 and GDF11 localized in neuronal nuclei might be important to inhibit neuronal proliferation via transcriptional regulation.

GDF8 and GDF11 expression in axons

In the present study, we detected GDF8 and GDF11 in most axons in the spinal trigeminal tract. As these axons are originated from primary afferent neurons situated in the trigeminal ganglion, the first explanation is that GDF8 and GDF11 produced in primary afferent neurons are anterogradely transported to the axon terminals. In contrast, in mammalian species, target-derived retrograde BMP signaling has been also reported to regulate trigeminal sensory neuron identities [13], and to determine the number of trigeminal ganglion [21]. Thus the second explanation might be that these GDF8 and GDF11 are up-taken at the axonal terminal and retrogradely-transported.

Conclusions

We investigated GDF8 and GDF11 expression in the adult rat trigeminal nuclei using immunohistochemistry. GDF8 and GDF11 were intensely and differentially expressed throughout the trigeminal nuclei. These data indicate that GDF8 and GDF11 play pivotal roles in the adult trigeminal system.

Conflict of interest statement

None declared.

Acknowledgment

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Table 1. Distribution and intensity of GDF8 and GDF11 in the trigeminal nuclei.

Area		GDF8	GDF11
Me5	nuclei	++++ / ++	+++
	cytoplasm	+	+++
	neuropil	+	++
Mo5	nuclei	++++	+++
	cytoplasm	++	+++
	neuropil	+	+
Pr5	nuclei	+++	+++
	cytoplasm	++	+++
	neuropil	+	+
Sp5O	nuclei	+++	+++
	cytoplasm	+	+++
	neuropil	+	+
Sp5C	nuclei	+++	+++
	cytoplasm	+	+++
	neuropil	+	++
Par5	nuclei	+++	++++
	cytoplasm	++	++++
	neuropil	++	+++

Relative intensities were estimated by visual comparison of immunostained slide: +, low; ++, moderate; +++, strong; ++++, very strong.

Fig. 1.

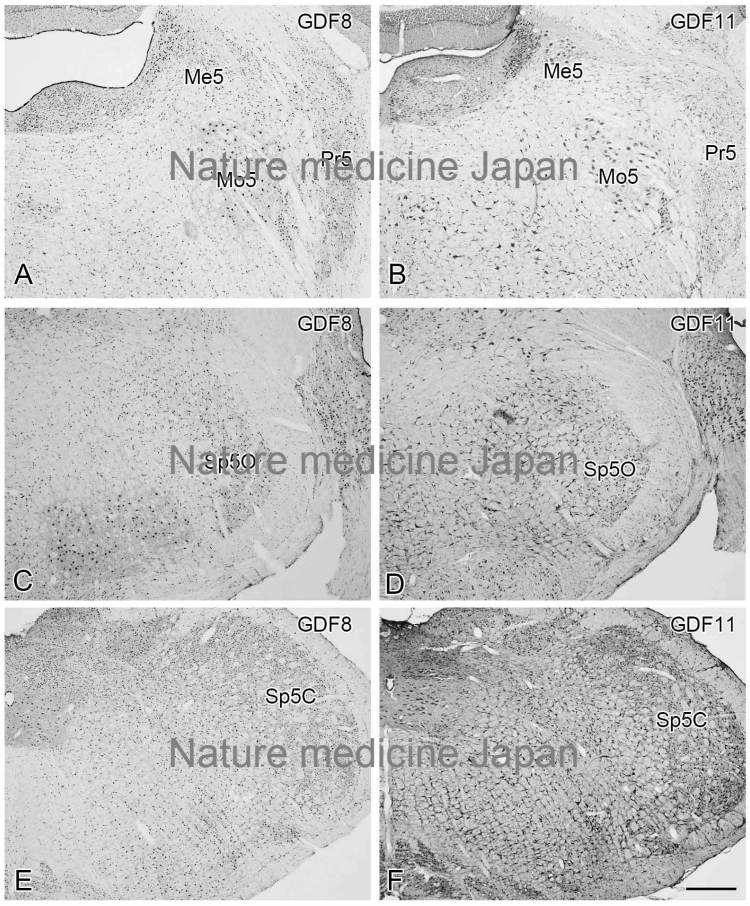

Fig. 1. GDF8 and GDF11 expression in the adult rat trigeminal nuclei. Me5, mesencephalic trigeminal nucleus; Mo5, motor trigeminal nucleus; Pr5, principal trigeminal nucleus; Sp5C, caudal part of spinal trigeminal nucleus; Sp5O, oral part of spinal trigeminal nucleus. Scale bar = 400 μm.

Fig. 2.

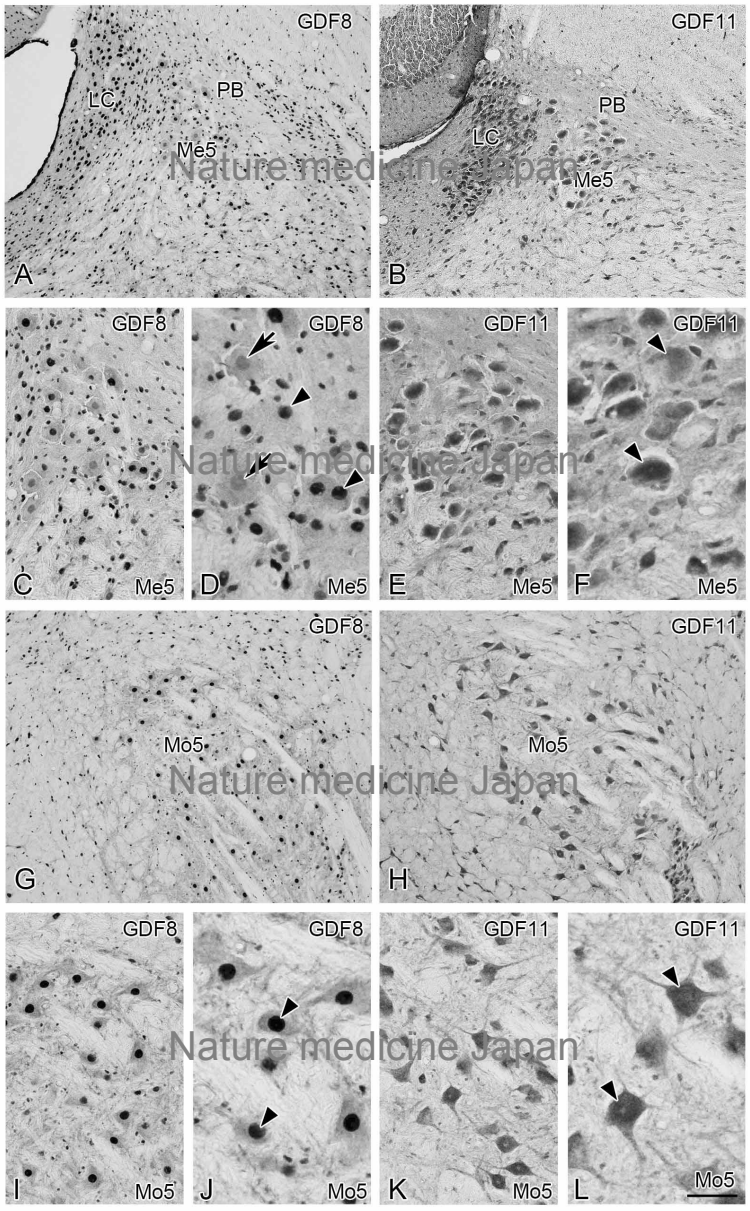

Fig. 2. GDF8 and GDF11 expression in the mesencephalic trigeminal nucleus (A-F) and the motor trigeminal nucleus (G-L). Note that GDF8-IR was strongly seen in nuclei (arrowheads in D), while GDF11-IR was detected in both nuclei and cytoplasm (arrowheads in F) in the mesencephalic trigeminal nuclues. LC, locus coeruleus; Me5, mesencephalic trigeminal nucleus; Mo5, motor trigeminal nucleus; PB, parabrachial nuclei. Scale bar = 160 μm for A, B, G, H; 80 μm for C, E, I, K; 40 μm for D, F, J, L.

Fig. 3.

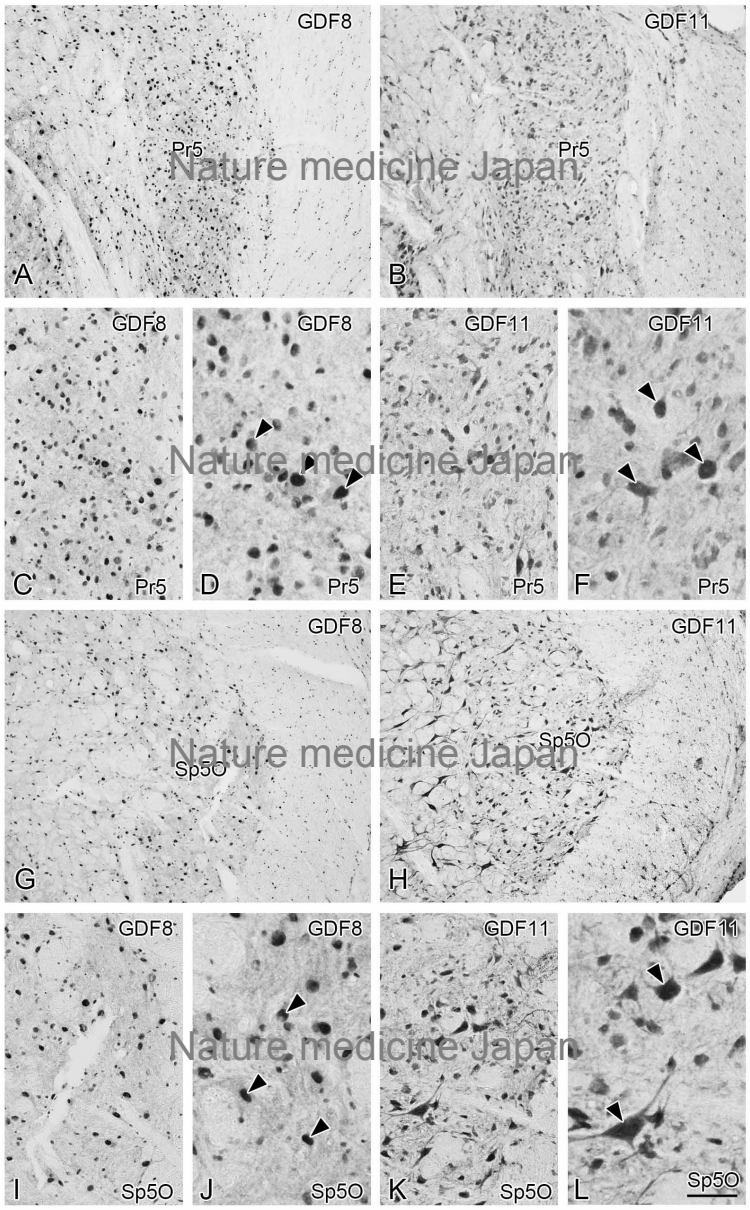

Fig. 3. GDF8 and GDF11 expression in the principal trigeminal nucleus (A-F) and the oral part of the spinal trigeminal nucleus (G-L). Note that abundant GDF8 and GDF11-IRs were seen in the principal trigeminal nucleus (A-F) and oral part of spinal trigeminal nucleus (G-L). Pr5, principal trigeminal nucleus; Sp5O, oral part of spinal trigeminal nucleus. Scale bar = 160 μm for A, B, G H; 80 μm for C, E, I, K; 40 μm for D, F, J, L.

Fig. 4.

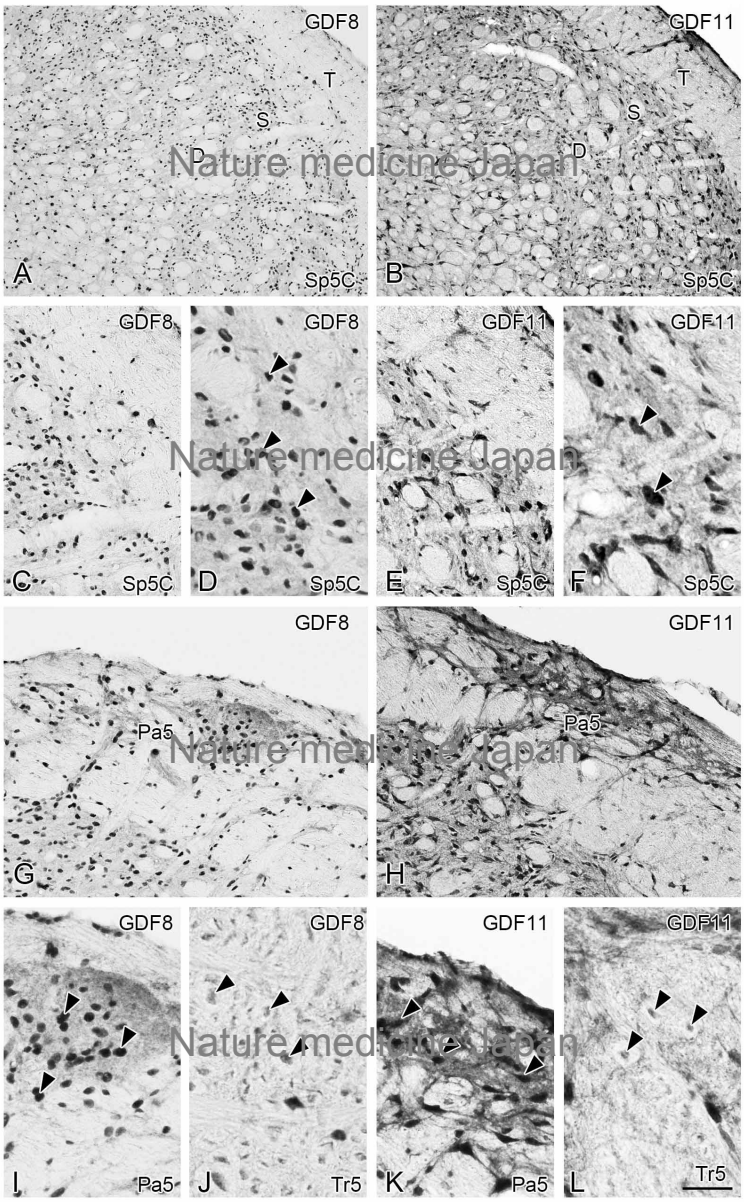

Fig. 4. GDF8 and GDF11 expression in the caudal part of the spinal trigeminal nucleus (A-F), the paratrigeminal nucleus (G-I and K), and the trigeminal tract (J and L). Note that GDF8 and GDF11-IRs were observed in many neurons in the caudal part of trigeminal spinal nucleus (A-F) and paratrigeminal nucleus (G-I and K). In addition, many axons in the trigeminal tract were positive for both GDF8-IR and GDF11-IR (arrowheads in J and L). D, deep layer; Pa5, paratrigeminal nucleus; S, superficial layer; Sp5C, caudal part of spinal trigeminal nucleus; t, tract; Tr5, trigeminal tract. Scale bar = 160 μm for A, B; 80 μm for C, E, G, H; 40 μm for D, F, I, K; 16μm for J, L.