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Nerve Growth Factor Involves Mutual Interaction between Neurons and Satellite Glial Cells in the Rat Trigeminal Ganglion

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Nerve growth factor (NGF) plays a critical role in the trigeminal ganglion (TG) following peripheral nerve damage in the oral region. Although neurons in the TG are surrounded by satellite glial cells (SGCs) that passively support neural function, little is known regarding NGF expression and its interactions with TG neurons and SGCs. This study was performed to examine the expression of NGF in TG neurons and SGCs with nerve damage by experimental tooth movement. An elastic band was inserted between the first and second upper molars of rats. The TG was removed at 0–7 days after tooth movement. Using *in situ* hybridization, NGF mRNA was expressed in both neurons and SGCs. Immunostaining for NGF demonstrated that during tooth movement the number of NGF-immunoreactive SGCs increased significantly as compared with baseline and reached maximum levels at day 3. Furthermore, the administration of the gap junction inhibitor carbenoxolone at the TG during tooth movement significantly decreased the number of NGF-immunoreactive SGCs. These results suggested that peripheral nerve damage may induce signal transduction from neurons to SGCs via gap junctions, inducing NGF expression in SGCs around neurons, and released NGF may be involved in the restoration of damaged neurons.

Key words: nerve growth factor, neurons, satellite glial cells, trigeminal ganglion, gap junction

I. Introduction

Nerve growth factor (NGF), a prototypical member of the neurotrophin family, is a candidate molecule that activates neurons following its secretion from satellite glial cells (SGCs) in the dorsal root ganglion (DRG) [22]. NGF plays essential roles in the survival and differentiation of sensory neurons. NGF plays a role in sensory neuron responses after peripheral nerve injury, including phenotype maintenance, sprouting, and regeneration [15, 20]. The actions of NGF on responsive cells are initiated by its binding to receptor molecules; NGF binds with low affinity to the p75 neurotrophin receptor (p75NTR) and with high affinity to the tyrosine kinase A (TrkA) receptor [16]. However, the expression of NGF in neurons or SGCs in sensory ganglia remains controversial. One study indicated that NGF mRNA is normally undetectable in sensory ganglia, and that NGF protein is synthesized in the target tissue and transported retrogradely to the responsive sensory neurons [12]. In contrast, Lee *et al.* [13] and Li *et al.* [14] found that NGF mRNA is expressed in neurons and SGCs of sensory ganglia. Zhou *et al.* [23] suggested that NGF plays an autocrine or paracrine role in DRG. Therefore, it is necessary to clarify whether NGF is expressed in the trigeminal ganglion (TG) neurons and/or SGCs.

The TG plays an important role in orofacial pain transmission. In the trigeminal somatosensory system, peri-

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odontal tissues in the maxilla and mandible are mainly innervated by primary sensory neurons in the TG. During experimental tooth movement, the branches of the trigeminal nerve are particularly vulnerable to inflammatory or injury responses in periodontal tissues. In response to oral nociceptive stimulation, signals are transmitted to neurons in the TG from peripheral tissue. Each neuronal cell in the sensory ganglion is tightly wrapped by a layer of SGCs [9]. SGCs directly influence neuronal activity by controlling the microenvironment in the ganglion [17]. Capuano *et al.* [4] reported that SGCs, in addition to providing mechanical and metabolic support to neurons, directly modulate their functions. Recent research has illuminated the complex, mutual communication between SGCs and neurons [5, 11]. In addition, neuronal activity plays an important role in the activation of glial cells in the DRG [21]. Furthermore, a major component regulating the perineuronal ionic environment is the coupling between adjacent SGCs via gap junctions, which allows the rapid transcellular exchange of small molecules. Gap junctions are channels that are formed by members of the connexin family of proteins that allow the direct passage of ions and other small molecules (<1 kDa) from one cell to another [1]. This coupling is dependent on physiological and pathological conditions, and the numbers of gap junctions between SGCs increase markedly in response to nerve injury [8, 10]. The existence of gap junctions between neurons and SGCs in the TG was indicated by the distribution of connexins [6], whereas Suadicani *et al.* [18] reported that calcium signaling from SGCs to neurons and among SGCs was reduced in the presence of the broad-spectrum P2 receptor (ATP receptor) antagonist suramin or the gap junction blocker carbenoxolone. In contrast, suramin, but not carbenoxolone, reduced signaling from neurons to SGCs. Therefore, we postulated that the glial gap junctions between neurons and surrounding SGCs are involved in the signal transduction from neurons to SGCs.

The present study was performed to investigate the expression of NGF in TG neurons and SGCs with degeneration of the nerve fibers of the periodontal membrane on the pressure side and stretching and tearing of nerve fibers on the tension side associated with orthodontic tooth movement. First, we demonstrated that NGF mRNA is expressed in neurons and SGCs in the TG using *in situ* hybridization. Second, we investigated the temporal changes in NGF expression in neurons and SGCs after application of experimental tooth movement. Finally, we examined the changes in NGF expression in the presence of the gap junction inhibitor carbenoxolone, which blocked the gap junctions between neurons and SGC after tooth movement.

II. Materials and Methods

Animals

All experimental protocols involving rats were reviewed and approved by the Animal Care Committee of Kyushu Dental University (Permission number: 10-010). Fifty-eight male Sprague-Dawley rats weighing 200–250 g each were used. The animals were acclimated for at least 1 week prior to the start of the experiment.

Experimental tooth movement

The rats were anesthetized with an intramuscular injection of ketamine (70 mg/kg; Daiichi Pharmaceutical, Tokyo, Japan) and xylazine (13 mg/kg; Bayer, Tokyo, Japan). An elastic band was inserted between left maxillary first and second upper molars. The rats treated were fed powdered food. Untreated animals were used as controls.

In situ hybridization

To examine the localization of NGF mRNA, normal rats (n=6) were anesthetized with diethyl ether and perfused transcardially with 4% paraformaldehyde (PFA) in 0.2 M phosphate buffer. The TG and submandibular gland, as a positive control, were then removed and postfixed in the same fixative overnight. The tissues were rapidly frozen and then cut into sections 6 μm thick using a cryostat. The frozen sections were hybridized with digoxigenin-labeled antisense riboprobes corresponding to partial cDNA of NGF. Plasmid DNA was a gift from Dr. Michael W. Miller (State University of New York-Upstate Medical University) [3] (Table 1). Using T7 or SP6 RNA polymerase (Invitrogen, Carlsbad, CA, USA), antisense and sense riboprobes were transcribed *in vitro* in the presence of DIG-labeled nucleotides (Roche Applied Science, Indianapolis, IN, USA). Sections were fixed in 4% PFA for 5 min and washed in phosphate-buffered saline (PBS, pH 7.4). For partial proteolysis, sections were permeabilized with 1 μg/ml proteinase K in 0.1 M PBS at 37°C for 10 min, followed by a 5-min wash in PBS. The sections were fixed again in 4% PFA for 10 min. For neutralization, sections were washed in 0.2% glycine in PBS for 5 min. Endogenous alkaline phosphatase activity was quenched with 0.2 N HCl for 30 min and washed in PBS for 5 min. After prehybridization for 5 min, hybridization was carried out at high stringency (1.3× standard saline citrate (SSC), 50% formamide, 57°C). Sections were washed in decreasing concentrations of SSC as follows: 5× SSC at room temperature (RT) for 30 min, 5× SSC/50% formamide at 50°C for 1 hr, 2× SSC at 50°C for 1 hr, and 0.2× SSC at 50°C for 1 hr. The sections were incubated at RT in 1%

Table 1. *Forward (F) and reverse (R) primers used to generate riboprobes for the in situ hybridization*

Gene	GenBank ID	Corresponding bases	Primer sets (5'→3')	cDNA fragment length (bp)
NGF	XM227525	269-801	F: GCGTAATGTCCATGTTGTTCTAC R: GGCTGTATCTATCCTGATGAACC	532

blocking buffer (Roche Applied Science) for 1 hr, followed by reaction with anti-alkaline phosphatase antibody at 1:500 dilution in 1% blocking buffer at 4°C overnight. For detection, signals were developed using nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) substrate (Roche Applied Science). Negative controls for *in situ* hybridization were carried out using sense probes.

Real-time polymerase chain reaction

Control rats (n=5) and experimental rats at 2 and 4 days after tooth movement (n=11) were killed by sodium pentobarbital injection. The TG of the experimental side was removed, homogenized in a Nippi BioMasher II (Nippi, Tokyo, Japan) with TRIzol Reagent (Invitrogen), and allowed to stand for 5 min at room temperature. Total RNA was dissolved in RNase-free water. The samples were then treated with DNase. Total RNA was reverse transcribed with random primers using a Superscript first-strand kit (Invitrogen). Real-time PCR was performed using the TaqMan Gene Expression Assay (Applied Biosystems, Foster City, CA, USA) and Eco Real Time PCR system (Illumina, San Diego, CA, USA). The TaqMan primer ID was NGF Rn01533872_m1. The level of target gene expression was normalized relative to that of β-actin and expressed relative to the control. The experiment was performed five times in each group.

Cannula implant for injection in the maxillary division of the trigeminal ganglion

The skull was exposed and a burr hole was drilled above the maxillary division of the left TG 6.5 mm anterior to interaural zero and 2.3 mm lateral to the midline. A guide cannula was fixed to the skull over the burr hole using two stainless steel screws and dental quick cure resin. The guide cannula extended into the burr hole 1 mm below the pedestal, but did not touch the surface of the cortex. At least 7 days were allowed for recovery from surgery before injection into the TG. After 7 days, each animal was given an injection of 10 μM carbenoxolone (LKT Laboratories, St. Paul, MN, USA), a gap junction-specific blocker, or normal saline. Before the injection, the rats were lightly anesthetized with isoflurane. A 24-gauge beveled stainless steel cannula was inserted through the guide cannula (positioned over the maxillary division of the left TG as described above) to 9.5 mm below the cortical surface. The injection cannula was connected to a 200-μl osmotic pump (ALZET mini-osmotic pump, model 2001; Alza, Palo Alto, CA, USA) attached to a microinjection pump set to deliver 1.0 μl over a period of 1 hr. The rats (n=11) had an elastic band inserted between their first and second upper molars. Cannulated rats were sacrificed on the third day after carbenoxolone injection. The experiment was performed five times in each group.

Tissue preparation

One, 3, 5, and 7 days after tooth movement, the rats (n=25, including the controls; the experiment was performed five times in each group) were anesthetized with diethyl ether and perfused transcardially with 4% PFA in 0.2 M phosphate buffer containing 0.2% picric acid. The TG of the experimental side was then removed and post-fixed in the same fixative overnight.

Immunohistochemistry

The TG was frozen rapidly and then cut into sections 6 μm thick using a cryostat (Leica Instruments, Wetzlar, Germany). Immunohistochemical staining with double-labeling for NGF and glutamine synthetase (GS), a satellite glial cell marker, was performed as described below. First, the sections were preincubated in 0.1 M PBS with 1% normal goat serum (ICN Pharmaceuticals, Costa Mesa, CA, USA) for 30 min at room temperature. The sections were incubated with sheep polyclonal antibodies against NGF (1:100; Abcam, Cambridge, MA, USA) for 2 hr at 37°C. After rinsing with 0.1 M PBS, the sections were incubated with FITC-conjugated donkey anti-sheep IgG (1:400; Molecular Probes, Eugene, OR, USA) for 2 hr at 37°C. After rinsing with 0.1 M PBS, the sections were incubated with rabbit polyclonal antibodies against GS (1:200; Sigma, St. Louis, MO, USA) washed in PBS, and incubated with TRITC-conjugated goat anti-rabbit IgG (1:400; Molecular Probes) for 2 hr at 37°C. Negative control sections were incubated with secondary antibody in buffer alone. The sections were washed in 0.1 M PBS and covered with coverslips.

Cell count

Images were acquired using a BIOREVO BZ-9000 fluorescence microscope (Keyence, Osaka, Japan). Ten randomly selected sections were obtained from each group. Images were obtained from two non-overlapping areas of each section. The size of one field was 221×166 μm, and each field included approximately 40 cells. The images were obtained at a standard exposure time across the slides. The images were analyzed quantitatively using NIH ImageJ software (NIH, Bethesda, MD, USA). Positive SGCs were identified based on a pixel intensity value over the threshold (background pixel intensity ±5 SD). Individual neurons were selected with the freehand outline tool in ImageJ on a pen tablet (XP-8060A; Active, Taipei, Taiwan). The outlined neurons were saved, and measurements were taken in the neuron area. The total number of neurons enclosed by immunopositive neurons and neurons surrounded by positive SGCs in the maxillary nerve region was determined for each section. Only those neurons with visible nuclei were counted.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the ratio of neurons with NGF-immunoreactive (NGF-IR) neurons and neurons surrounded by NGF-IR SGCs, followed by individual *post hoc* comparisons (Scheffé's test).

III. Results

Expression of NGF mRNA

To determine whether NGF mRNA is expressed in the neurons and SGCs of the TG, we performed *in situ* hybridization in specimens from untreated rats. The expression of NGF mRNA was confirmed in the submandibular gland as a positive control with the antisense probe (Fig. 1A). In TG, NGF mRNA was expressed in both neurons and the SGCs surrounding the neurons (Fig. 1C, D). No labeling was found with the NGF sense probe (Fig. 1B, E).

NGF mRNA expression in the trigeminal ganglion after tooth movement

The expression levels of NGF mRNA in the TG after tooth movement were analyzed by real-time PCR. Expression of NGF mRNA was significantly increased on day 2 compared with controls and then decreased on day 4 after tooth movement (Fig. 2).

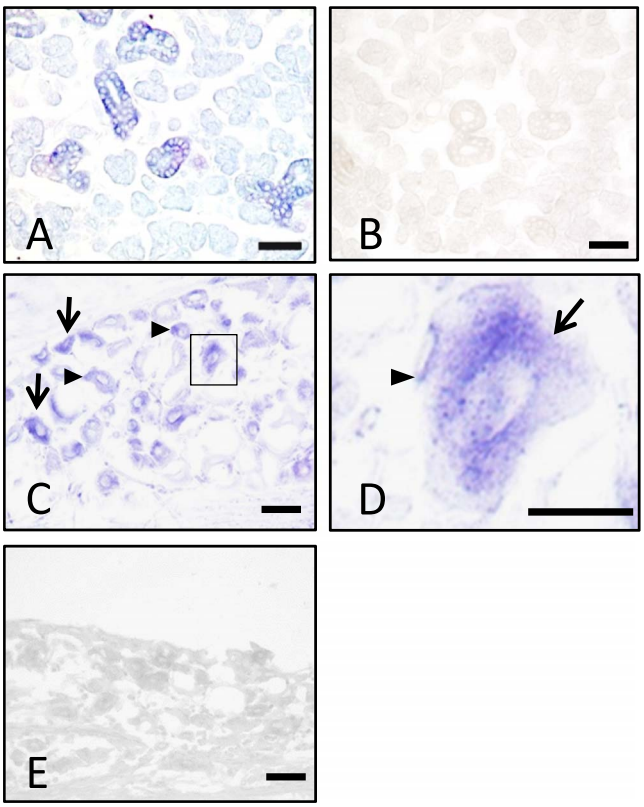

Fig. 1. NGF mRNA expression in the submandibular gland and trigeminal ganglion (TG) of normal rats. *In situ* hybridization for NGF mRNA in the submandibular gland with the antisense probe (**A**) and sense probe (**B**). (**C**) The neurons (arrow) and satellite cells (arrowhead) showed NGF mRNA expression. (**D**) Higher magnification of the highlighted region in (**C**). Strong signals for NGF mRNA were observed in the neurons (arrow) and satellite cells (arrowhead). (**B**, **E**) *In situ* hybridization for NGF mRNA with sense probe showing no positive staining. Bars=50 μm (**A**, **B**), 30 μm (**C**, **E**), and 10 μm (**D**).

NGF-IR neurons and SGCs after tooth movement

Temporal changes in the NGF-IR neurons and SGCs were examined in the maxillary nerve region of the TG. We used GS as a marker for SGCs. Three types of NGF-IR were observed: the neuron was immunopositive for NGF, but surrounding SGCs were negative [N(+)S(–), Fig. 3A]; both the neuron and surrounding SGCs were positive for NGF [N(+)S(+), Fig. 3B]; and the SGCs were positive for NGF, but the neuron they surrounded was negative [N(–)S(+), Fig. 3C]. For NGF-IR neurons, we measured both N(+)S(–) and N(+)S(+) types. For NGF-IR SGCs, we measured the number of neurons surrounded by NGF-IR SGCs [*i.e.*, N(–)S(+) and N(+)S(+) types] rather than counting the number of NGF-IR SGCs.

In the untreated controls, about 36.2% of the neurons were NGF-IR, and about 23.8% of the neurons were surrounded by NGF-IR SGCs (Fig. 4A–C). One day after tooth movement, the number of NGF-IR neurons [N(+)S(–) and N(+)S(+)] and the number of neurons surrounded by NGF-IR SGCs [(N(–)S(+)] were increased slightly as compared with controls (NGF-IR neurons: 38.1% of the neurons in the maxillary nerve region; neurons surrounded by NGF-IR SGCs: 27.8% of the neurons in the maxillary nerve region) (Fig. 4D–F). Three days after tooth movement, the number of NGF-IR neurons was decreased, while the number of neurons surrounded by NGF-IR SGCs was increased significantly as compared with controls (NGF-IR neurons: 26.6% of the neurons in the maxillary nerve region, neurons surrounded by NGF-IR SGCs: 57.9% of the neurons in the maxillary nerve region) (Fig. 4G–I). Five days after tooth movement, the number of NGF-IR neurons was then increased, while neurons surrounded by NGF-IR SGCs were decreased as compared with 3 days after tooth movement (NGF-IR neurons: 50.9% of the neurons in the maxillary nerve region; neurons surrounded by NGF-IR SGCs: 37.1% of the neurons in the maxillary nerve region) (Fig. 4J–L). Seven days after tooth movement, the number of NGF-IR neurons had not changed as compared with the

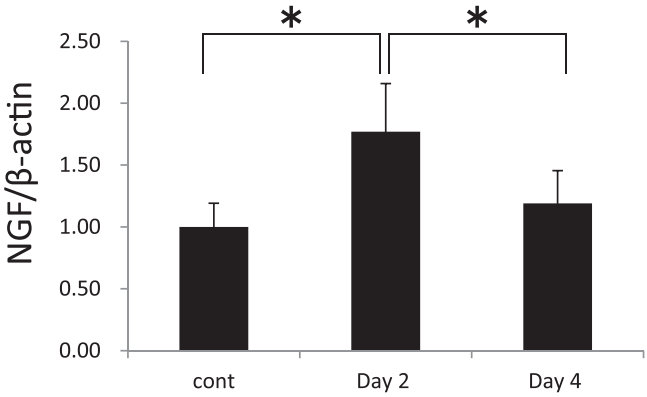

Fig. 2. Temporal changes in the expression of NGF mRNA in the trigeminal ganglion (TG) after tooth movement. Results of quantitative real-time polymerase chain reaction analysis for NGF mRNA expression. The mRNA levels are expressed relative to the control. Data represent means±SD. **P*<0.01.

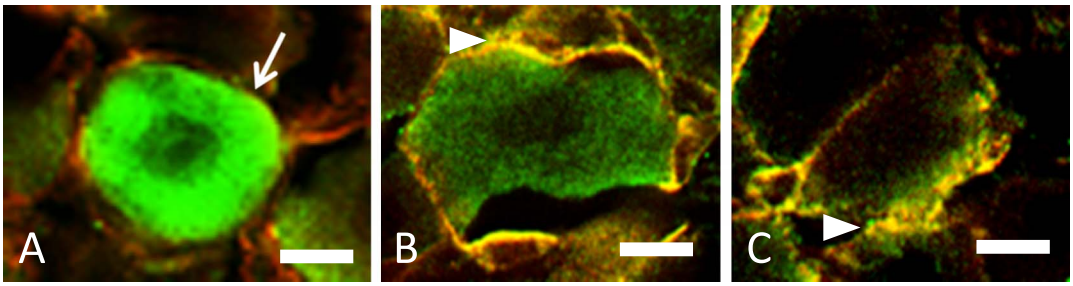

Fig. 3. NGF-IR at satellite glial cells (SGCs) or neurons in the trigeminal ganglion (TG). Three types of NGF-IR were observed: (**A**) the neuron was immunopositive for NGF (arrow), but surrounding SGCs were negative [N(+)S(–)]; (**B**) both the neuron and surrounding SGCs (arrowhead) were positive [N(+)S(+)]; and (**C**) the SGCs were positive, but the neuron they surrounded was negative [N(–)S(+)]. Green; NGF-IR cells, red; GS-immunoreactive cells, and yellow; NGF and GS double positive cells. Bars=10 μm (**A**, **B**, and **C**).

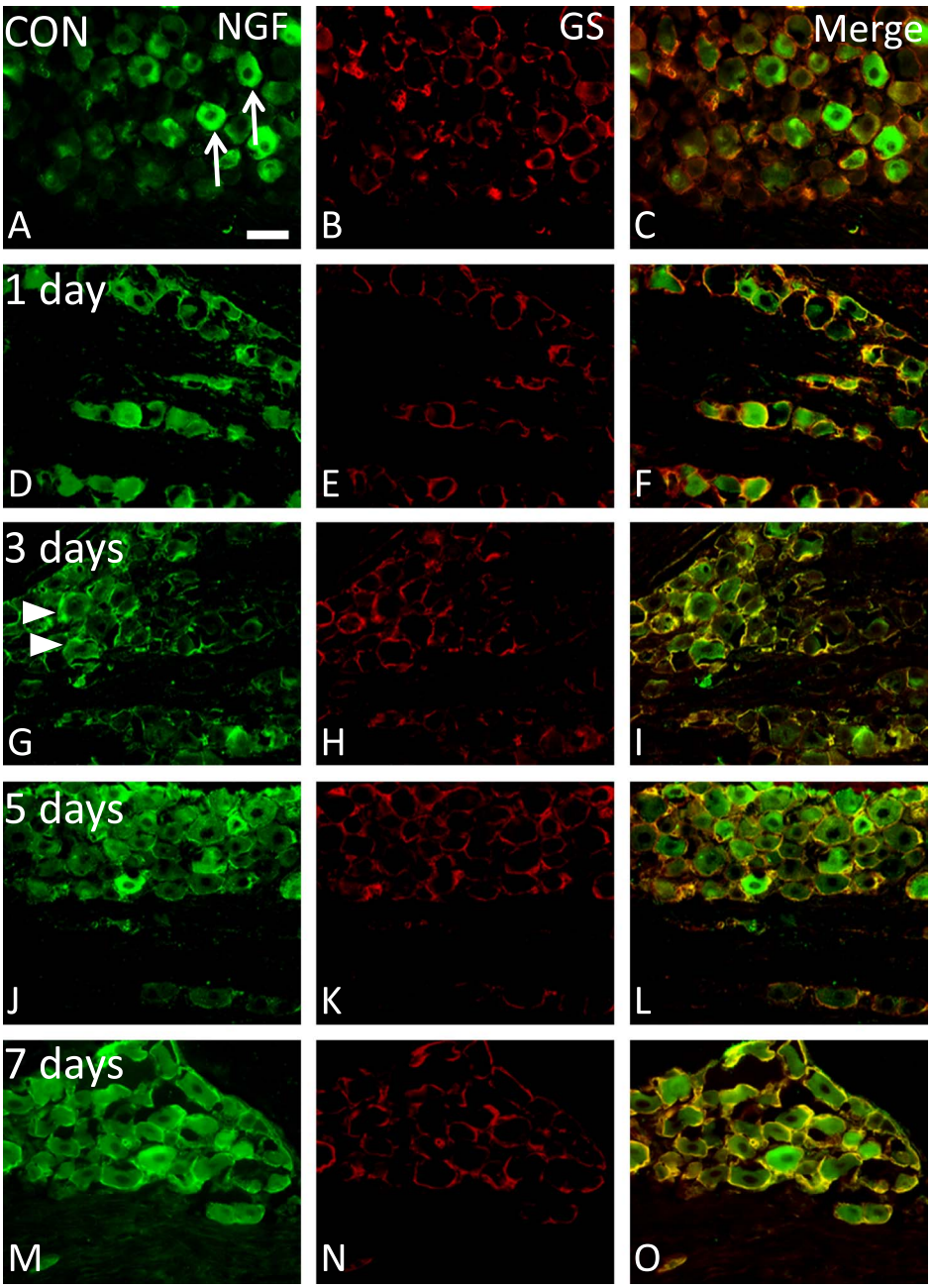

Fig. 4. Distribution of NGF-IR cells and GS-immunoreactive (GS-IR) satellite cells in the maxillary nerve region of the trigeminal ganglion. NGF-IR cells (**A**, **D**, **G**, **J**, and **M**) and GS-IR satellite cells (**B**, **E**, **H**, **K** and **N**) in control rats (**A** and **B**) and in rats at 1 (**D** and **E**), 3 (**G** and **H**), 5 (**J** and **K**), and 7 days (**M** and **N**) after tooth movement. (**C**, **F**, **I**, **L**, and **O**) are merged images of (**A**, **D**, **G**, **J**, and **M**) and (**B**, **E**, **H**, **K**, and **N**). Arrows in (**A**): NGF-IR neurons; arrowheads in (**G**): NGF-IR satellite cells. Bars=50 μm (**A**).

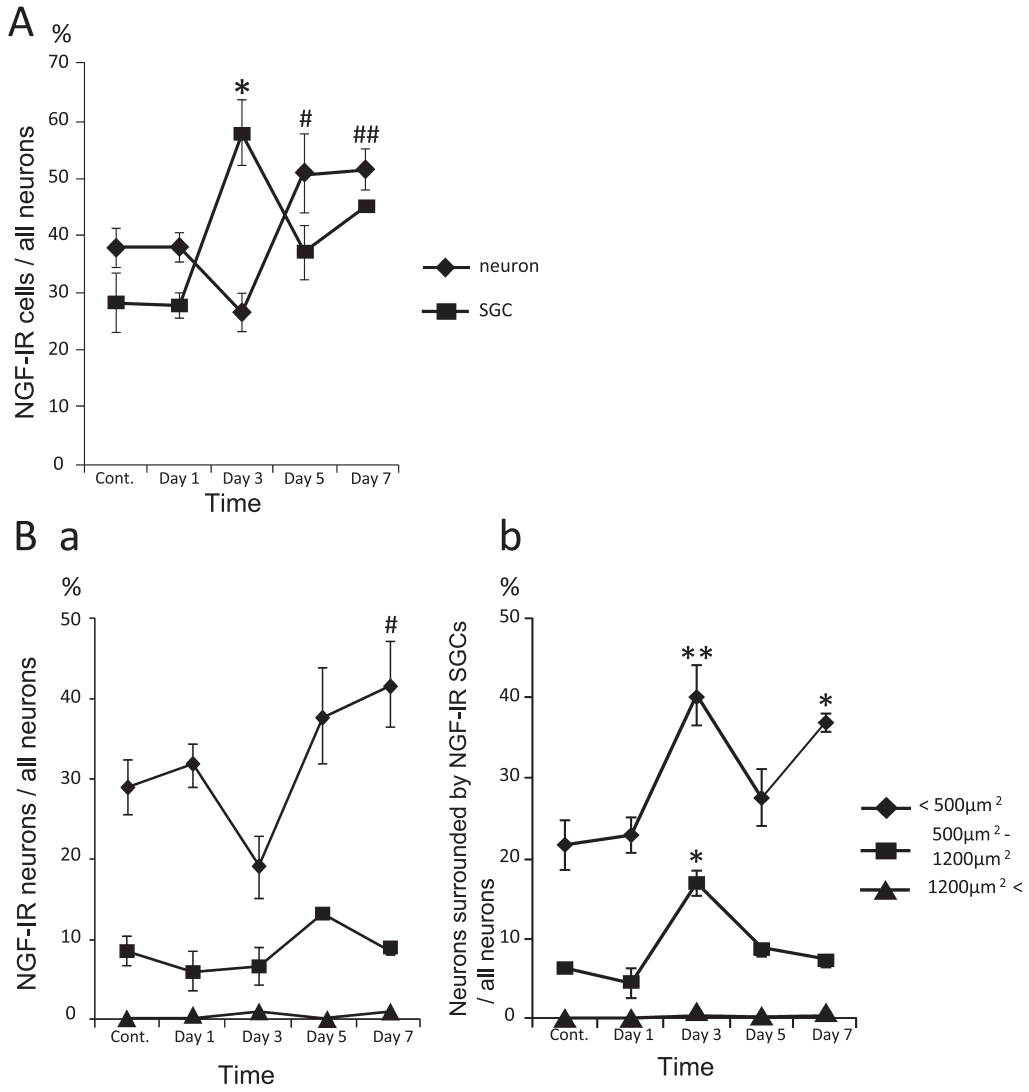

Fig. 5. The ratio of NGF-IR neurons and neurons surrounded by NGF-IR satellite cells in the trigeminal ganglion. (**A**) The ratio of NGF-IR neurons and neurons surrounded by NGF-IR satellite cells per all neurons in the maxillary nerve region. (**B**) Ratios of small (<500 μm^2), medium (500–1,200 μm^2), and large (>1,200 μm^2) NGF-IR neurons per all neurons in the maxillary nerve region (**a**). The ratio of small (<500 μm^2), medium (500–1,200 μm^2), and large (>1,200 μm^2) neurons surrounded by NGF-IR satellite cells per all neurons in the maxillary nerve region (**b**). The results are the means±SEM. Control group n=4. Experimental group n=21. *P<0.05 **P<0.01 compared with control. #P<0.05, and ##P<0.01 compared with 3 days after the start of experimental tooth movement.

fifth day, while neurons surrounded by NGF-IR SGCs were increased slightly (NGF-IR neurons: 51.6% of the neurons in the maxillary nerve region; neurons surrounded by NGF-IR SGCs: 45.2% of the neurons in the maxillary nerve region) (Fig. 4M–O).

The number of NGF-IR neurons was decreased on day 3 and then increased on day 5, whereas the number of neurons surrounded by NGF-IR SGCs was increased on day 3 and then decreased on day 5 (Fig. 5A). Next, we examined the size-dependent changes in NGF-IR neurons. Both the NGF-IR neurons and the neurons surrounded by NGF-IR SGCs were mainly small (<500 μm^2) or medium (500–1,200 μm^2) in size (Fig. 5Ba, b). The ratio of small NGF-IR neurons decreased on day 3 and then increased on day 5, whereas the ratio of medium and large NGF-IR neurons remained constant (Fig. 5Ba). The ratio of small and medium-size neurons surrounded by NGF-IR SGCs was increased on day 3 and then decreased on day 5 (Fig. 5Bb).

NGF-IR neurons and SGCs after tooth movement when gap junctions were inhibited

We examined whether blocking of gap junctions with 10 μM carbenoxolone would alter the expression of NGF in neurons and SGCs after tooth movement. In the group injected with normal saline, the numbers of NGF-IR neurons and neurons surrounded by NGF-IR SGCs were similar to those in the no-administration group 3 days after tooth movement (Fig. 6A–C vs. Fig. 4G–I). However, 3

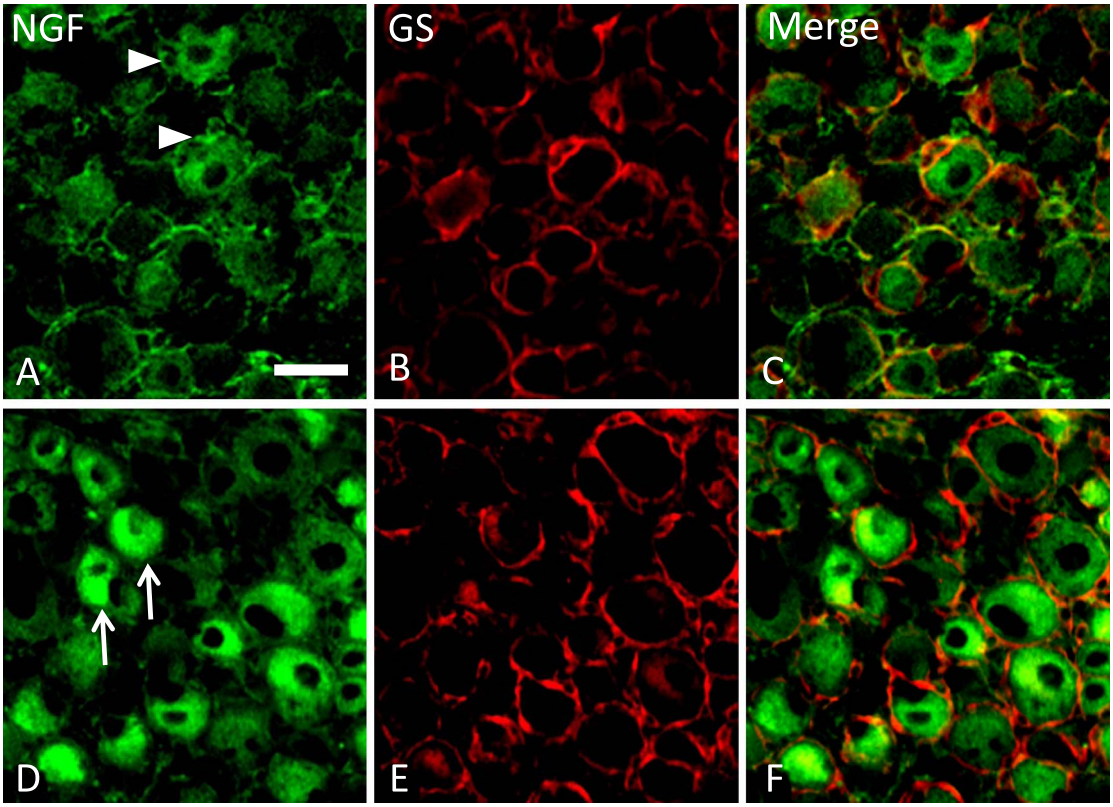

Fig. 6. The effects of carbenoxolone on the distribution of NGF-IR cells and GS-IR satellite cells. NGF-IR neurons and GS-IR SGCs in the maxillary nerve region of the TG 3 days after experimental tooth movement were stained using specific antibodies against NGF and GS, respectively. NGF-IR cells (**A** and **D**) and GS-IR satellite cells (**B** and **E**) in rats at 3 days. (**C** and **F**) are merged images of (**A** and **D**) and (**B** and **E**), respectively. (**A–C**) The rats were administered saline. (**D–F**) The rats were administered carbenoxolone. Arrowheads in (**A**): NGF-IR satellite cells; arrows in (**D**): NGF-IR neurons. Bars=30 μm (**A**).

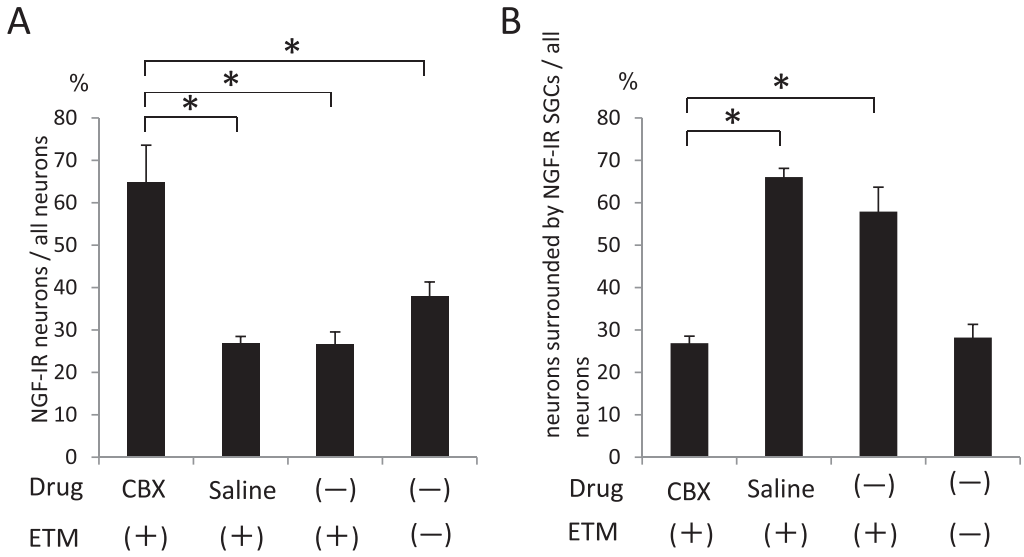

Fig. 7. The effects of carbenoxolone on the ratio of NGF-IR neurons and neurons surrounded by NGF-IR satellite cells at 3 days after experimental tooth movement (ETM). (**A**) The ratio of NGF-IR neurons to all neurons in the maxillary nerve region. (**B**) The ratio of neurons surrounded by NGF-IR satellite cells to all neurons in the maxillary nerve region. The results are the means±SEM. **P*<0.01 as compared with [Drug (+), ETM (+)].

days after tooth movement, NGF-IR neurons were increased and neurons surrounded by NGF-IR SGCs were decreased as compared with the no-administration group (Fig. 6D–F).

Upon administration of carbenoxolone, the ratio of NGF-IR neurons to all neurons in the maxillary nerve region was significantly greater as compared with the other three groups 3 days after tooth movement (NGF-IR neurons in the group administered carbenoxolone: 64.9%, NGF-IR neurons in the group administered saline: 26.9%) (Fig. 7A). The ratio of neurons surrounded by NGF-IR satellite cells to all neurons in the maxillary nerve region was significantly lower as compared with the other three groups 3 days after tooth movement (neurons surrounded by NGF-IR SGCs in the group administered carbenoxolone: 26.9%, neurons surrounded by NGF-IR SGCs in the group administered saline: 66.1%) (Fig. 7B).

IV. Discussion

In this study, we determined the changes in NGF expression patterns induced by damage to peripheral axons during experimental tooth movement and the functional interaction between neurons and SGCs in the nociceptive TG.

Although there is controversy regarding whether NGF is expressed in neurons or SGCs in sensory ganglia, we confirmed that NGF mRNA is expressed in the neurons and SGCs of rat TG using *in situ* hybridization and immunocytochemistry. The upregulation of NGF by nociceptive stimulation is consistent with the reported upregulation of NGF expression in DRG induced by damage to the peripheral axons [22]. After tooth movement, we expected that the expression of NGF mRNA in neurons and SGCs would increase. We found no significant increases in the NGF mRNA signal by *in situ* hybridization but detected time-dependent changes in NGF mRNA expression by real-time PCR. Our findings were consistent with those of Brown *et al.* [2], who reported that NGF mRNA expression was upregulated by damage to the central processes of sensory neurons in DRG.

The number of NGF-IR neurons decreased on day 3 and increased on day 5 following tooth movement. In contrast, the number of NGF-IR SGCs increased significantly and reached a maximum on day 3, decreased on day 5, and then increased again slightly on day 7. With regard to the decrease in NGF-IR neurons, the nociceptive stimulation caused by application of experimental tooth movement would initially induce the release of NGF from the neurons. However, we did not obtain any data indicating a release of NGF from the periphery of axons or cell bodies of neurons; therefore, further studies are needed to clarify the release of NGF from neurons. Gunjigake *et al.* [7] reported that nerve injury caused by extraction of the maxillary molars in rats induced the activation of SGCs around the injured neurons. In terms of the NGF-IR SGCs, we found an increase in NGF-IR SGCs located close to the damaged neurons. The increase in NGF-IR SGCs was likely caused by signal transduction from the damaged neuron to the surrounded SGCs.

The type of communication that occurs between SGCs and sensory neurons is controversial. In this study, we investigated the involvement of gap junctions between neurons and SGCs using the gap junction blocker, carbenoxolone, *in vivo* by direct cannulation to the TG. Blocking the gap junctions between neurons and SGCs reduced the expression of NGF in the SGCs, whereas the expression of NGF was increased in neurons 3 days after experimental tooth movement. As NGF itself cannot cross the gap junction, some signals that induce NGF production in SGCs may not be transferred from neurons to SGCs via the gap junctions. The mechanism underlying the effects of increased NGF-IR neurons on blocking gap junctions remains unclear. Other than the connexin-43 subunit of gap junctions, SGC-specific proteins such as the inwardly rectifying K^+ channel Kir4.1, the purinergic receptor P2Y4, and soluble guanylate cyclase [19] are associated with communication between neurons and SGCs. Furthermore, a previous study indicated that silencing SGC-specific molecules involved in intercellular transport (connexin-43) or glutamate recycling (glutamine synthase) can dramatically alter the nociceptive responses of normal and nerve-injured rats [11]. Xie *et al.* [21] reported that as NGF has excitatory effects on sensory neurons, this potentially represents another positive feedback loop between spontaneously active neurons and neighboring activated satellite glia cells. Taken together, these findings suggest that gap junctions between neurons and SGCs in the TG may play some role in reciprocal communication during the genesis, maintenance, and resolution of pain.

Finally, we investigated the size of the neurons that express NGF following peripheral damage. In this study, most of the NGF-IR neurons were small (<500 μm^2) or medium (500–1200 μm^2) in size, which suggests that they were mainly c-fiber unmyelinated neurons. As the damage to peripheral axons caused by tooth movement was moderate, it may cause dull pain to be transported through c-fiber unmyelinated neurons.

In conclusion, this study demonstrated that experimental tooth movement induced signal transduction from the damaged neurons to the surrounded SGCs via gap junctions. The SGCs produce the NGF that induces activation of the damaged neurons. These findings suggest that there is a positive feedback loop between damaged neurons and surrounding satellite glial cells in the TG.

V. Acknowledgments

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