Peripheral Brain-Derived Neurotrophic Factor Modulates Regeneration Following Inferior Alveolar Nerve Injury in Rats

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Aims: To examine the effects of local brain-derived neurotrophic factor (BDNF) produced after nerve injury on the functional regeneration of the damaged nerve. **Methods:** The inferior alveolar nerve was transected in adult male rats and 1 µg or 10 µg of BDNF antibody was administered at the injury site; a third group of rats received saline and a fourth group underwent nerve ligation. BDNF mRNA was quantified in the transected tissue and trigeminal ganglion by using real-time polymerase chain reaction (PCR). Head withdrawal thresholds following mechanical (tactile) stimulation (with von Frey filaments) of the mental region were measured for 3 weeks postoperatively. Electromyographic activity of the jaw opening reflex (JOR) was recorded from the anterior belly of the digastric muscle. **Results:** Within 24 hours, transection induced significant elevation of BDNF mRNA expression in the injured tissue (unpaired *t* test, $P < .01$). The head withdrawal threshold to mechanical stimulation increased at 1 day after transection and then decreased (two-way repeated measures analysis of variance [ANOVA], $P < .001$). At 2 weeks after surgery, the head withdrawal threshold was higher than before surgery in the group that received a higher dose of BDNF antibody (ANOVA, $P < .001$), but not in the group that received a smaller dose (ANOVA, $P > .05$). No significant differences were observed in the latency or threshold of the JOR between saline- and antibody-treated rats (unpaired *t* test, $P > .05$). **Conclusion:** These results suggest that locally administered BDNF antibody neutralizes nerve injury–induced BDNF at the injury site and thus influences sensorimotor recovery. *J Oral Facial Pain Headache 2016;30: 346–354. doi: 10.11607/ofph.1651*

Keywords: *jaw-opening reflex, neurotrophic factor, peripheral nerve injury, regeneration, withdraw reflex*

Brain-derived neurotrophic factor (BDNF) is widely distributed in the central and peripheral nervous systems and promotes neurite extension by ensuring myelination and neuronal survival.[1–4] BDNF exerts this function by binding to its high-affinity receptor, tyrosine receptor kinase B (TrkB).[5,6] The BDNF–TrkB system is involved in neuroregeneration after nerve damage[7–9] and promotes the extension of neurites in injured sensory neurons.[10,11] After regeneration of the injured nerve, dysesthesia can sometimes occur.[12,13] Peripheral nerve transection also induces changes in the head withdrawal threshold as response to mechanical or heat stimulation of the facial skin in animals.[14] Deprivation of endogenous BDNF causes inhibition of myelination while local administration of BDNF after denervation promotes neuroma formation, initiates autotomy, and increases autotomy severity.[15,16] These findings suggest that BDNF is involved in nerve regeneration following peripheral nerve damage.

It has recently been demonstrated that local administration of an antibody to BDNF at a trigeminal nerve injury site caused axonal reconnection and inhibited neuroma formation, suggesting that the neuronal regeneration mechanism may involve local BDNF.[17] However, little is known about the effects of BDNF on the recovery of sensory function after peripheral nerve injury. Therefore, the aim of this study was to examine the effects of local BDNF produced after nerve injury on the functional regeneration of the damaged nerve.

Materials and Methods

Animals

Experiments were approved by the Niigata University Intramural Animal Use and Care Committee, Niigata, Japan (Approval Number: 42). Male, 6-week-old Sprague-Dawley rats (Charles River Laboratories Japan) were housed at 25°C and approximately 40% humidity with a 12-hour light/dark cycle and free access to food and water.

Surgery and Grouping

The rats were deeply anesthetized by inhalation of sevoflurane and intraperitoneal injection of pentobarbital (50 mg/kg). The depth of anesthesia was monitored repeatedly during surgery by pinching the paws. The buccal aspect of the mandibular skin was incised to expose the surface of the mandible. A 3-mm length of the left inferior alveolar nerve (IAN) was then exposed and transected 5 mm distally to the mandibular foramen (5 mm from the mental foramen and 3 mm from the inferior border of the mandible).

Immediately after nerve transection, animals were allocated to one of four groups: A-B1, A-B10, normal saline (NS), or nerve ligation (LN). Rats in the A-B1 and A-B10 groups received either 1 µl (A-B1 group) or 10 µl (A-B10 group) of BDNF antibody (1 mg/ml, rabbit anti-BDNF polyclonal IgG, ab6201; Abcam) injected into the IAN lesion site by using a syringe (Hamilton). A hemostatic gelatin sponge (Sponge, Astellas Pharma) was placed on the transected region and bone wax (Angiotech Pharmaceuticals) was then used to cover the region to prevent leakage of the administered BDNF antibody solution.

Rats in the NS group received physiologic saline instead of BDNF antibody solution, and in the nerve ligation (LN) group the transected stump was ligated with silk thread and the gaps around the stump were filled with bone wax to prevent nerve reconnection after transection. Rats in the LN group were used to confirm the changes in sensorimotor response without regeneration or reconnection of transected nerve and so did not receive local administration of BDNF antibody or saline.

BDNF mRNA Expression

BDNF mRNA levels in the transected nerve were measured using real-time polymerase chain reaction (PCR). Under deep general anesthesia induced by sevoflurane inhalation, the transected nerve (including the surrounding tissue) and the ipsilateral and contralateral trigeminal ganglia were removed from the rat for analysis. Samples were obtained at 0 hours (pretransection) and 24 hours after IAN transection (n = 3 rats in each sample) and BDNF mRNA expression was compared between the two sides and the two time points.

Total RNA was isolated from the tissue samples using TRIzol Reagent (Life Technologies). Single-stranded cDNA was obtained using 0.5 µg RNA extracted by reverse transcription using a Perfect Real Time PrimeScript RT Reagent Kit (Takara Bio).

Quantitative real-time PCR was performed using an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) with Taq II (Perfect Real Time PrimeScript RT Reagent Kit). TIi RNaseH Plus SYBR PremixEx Taq II (Takara Bio) was added to the synthesized cDNA and the mixture was incubated at 95°C for 30 seconds. PCR was then conducted at 95°C for 5 seconds and 60°C for 30 seconds for 40 cycles. PCR was performed using rat BDNF- or β-actin–specific primers. β-actin was used as an internal standard and mRNA was quantified using the relative quantification method according to the ratio of BDNF and β-actin. The BDNF primers used were: forward, 5′-ATTACCTGGATGCCGCAAAC-3′ and reverse, 5′-TTTTATCTGCCGCTGTGACC-3′; expected size: 117 base pairs (bp). β-actin primers were: forward, 5′-CAGGGTGTGATGGTGGGTAT-3′ and reverse, 5′-GTGTGGTGCCAAATCTTCTC-3′; expected size: 146 bp.

Histopathologic Analysis

The transected area was observed in all groups (A-B1, A-B10, NS, and LN; n = 6 per group). In a previous study,[17] histopathologic analysis revealed signs of regeneration in the transected nerve 2 weeks after IAN injury. To further characterize the time course of changes in sensorimotor response during the regeneration process, the period of histopathologic observation in the present study was extended to 3 weeks after injury. Therefore, 3 weeks after IAN transection, all rats were placed under deep anesthesia with sevoflurane and perfused transcardially with physiologic saline, followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (pH 7.4). Each mandible, including the bone surrounding the transected IAN, was removed en bloc, decalcified in formic acid for 8 days, and dried. The tissue was embedded in paraffin and cut into 7-µm-thick sections. The specimens were stained with Heidenhain's Azan (azocarmine G and aniline blue) using the Mallory staining technique to visualize fibrous tissue and collagen from other tissues. Images were taken with an optical microscope and observed using Zeiss AxioVision microscopy software (Release 4.8; Carl Zeiss AG).

Measurement of Head Withdrawal Threshold

The head withdrawal threshold in response to mechanical stimuli at various intensities, applied to the mental region innervated by the transected nerve,

was measured using von Frey filaments (Touch Test Sensory Evaluators). Chronologic changes in the threshold were investigated and thresholds were compared between the four groups. Measurement of the head withdrawal threshold was performed according to the method of Iwata et al[18] with minor modifications. Rats received training for 1 week prior to surgery to habituate them to mechanical stimuli in the mental region. The animals were placed for 15 minutes a day in a plastic cage (6 × 11 × 5.5 cm) with a window (aperture diameter of 2.5 cm) allowing the snout to protrude. The protruded mental region was mechanically stimulated using a von Frey filament so that the rats became accustomed to mechanical stimulation of up to 10 g. At 1 week after habituation the IAN was transected as described above, and the head withdrawal threshold measurements were performed at 3, 7, 14, and 21 days postoperative (3 weeks) by using filaments of 0.6–60 g (the cut-off value was set at 60 g because excessive pressure can lead to head movement even if the animals do not sense the stimulation). The stimulus was applied from small to larger forces until a withdrawal response of the head was elicited, and the force at which the response was elicited was noted. Next, the stimulus was applied from large to smaller forces until the response disappeared, and the force at which the response was no longer elicited was noted. The head withdrawal threshold was defined as the mean of these two values. The measurements were performed three times each.

Jaw-Opening Reflex

Electromyographic (EMG) activity related to the jaw-opening reflex (JOR) was recorded from the anterior belly of the digastric muscle. Only the A-B1, NS, and LN groups were used for these measurements.

At 3 weeks after nerve transection, the femoral vein was catheterized for drug delivery. A mixture of urethane (500 mg/kg) and α-chloralose (50 mg/kg) was delivered to replace the isoflurane anesthesia before recording. The depth of anesthesia was checked repeatedly throughout the experiment by pinching the paws and was maintained at the level at which a small response remained present. If the paw pinch elicited a large withdrawal reflex, an additional dose of α-chloralose-urethane mixture was administered. During the measurements, rectal temperature was monitored and maintained at 37°C to 38°C with a heating pad.

The submandibular skin was incised and the anterior belly of the digastric muscle was exposed. Paired copper wires (0.12 mm in diameter, with 3-mm interpolar distance, and 1-mm tip exposure) were inserted bilaterally into the anterior belly of the digastric muscle to detect EMG activity. Paired Teflon-coated stainless steel wires (0.1 mm in diameter, with 0.5-mm tip exposure, 2-mm insertion, and an acquired difference of 1 mm) were inserted into the mental nerve at the mental foramen (4 mm distally to the transected site) to stimulate the IAN.

Pulse stimulation (single pulse, 0.2-millisecond duration) was applied to the mental nerve at 5-second intervals. The JOR threshold was determined as the minimum stimulus current that consistently evoked an EMG-detectable reflex response of this muscle. To measure the response latency and threshold, the stimulus current was set at 1.5 times the measured JOR threshold. Previous histologic observations of lesions in the LN group showed that the transected nerve ends did not appear to reconnect. Therefore, in the LN group, a current five times higher on the ligation side than on the contralateral (naïve) side was used to ensure that any previously unobserved regenerated pathway between the resected nerve ends was included. EMG activity was amplified using current amplifiers (bandpass: 0.1–3 kHz) and the signals were fed into a computer equipped with a CED Power1401 board and Spike2 analysis software (Cambridge Electronic Design). The sampling rate for the EMG activity was 5,000 samples/second. Recorded EMG activity was stored electronically and analyzed offline. The stimulus pulses for the test stimuli were also fed into a computer with a CED Power1401 board as event signals.

The JOR was evaluated by the measured mean latency and threshold. To assess latency, EMG signals were full-wave rectified and smoothed (time constant: 20 milliseconds) using Spike2. To define the onset of the reflex, baseline EMG activity was measured for 2 minutes during the control period, and the onset was defined as the time point at which the standard deviation (SD) of EMG activity exceeded two SD from baseline EMG activity. The latency was defined as the time from the first stimulus pulse to the onset of EMG activity.

Statistical Analyses

All data are presented as mean ± SD. Two-way repeated measures analysis of variance (ANOVA) was used to test for significant differences in the head withdrawal thresholds between the A-B1, A-B10, and NS groups, and post hoc analyses were performed using Tukey's method. In the LN group the head withdrawal threshold exceeded the cut-off value for the duration of the measurement period and was markedly higher than the other groups from 3 days postoperative to 3 weeks postoperative. Therefore, these values for the LN group were excluded from the statistical analyses. JOR latencies and thresholds were compared between the A-B1 and NS groups by using an unpaired *t* test. The LN group, for which the IAN could not regenerate after the injury, exhibited no

response to the stimulation throughout the observation period. Therefore, the data from this group were not used in the statistical analysis of the effect of the BDNF antibody on reflex recovery. Statistical differences in the quantity of mRNA in the tissues surrounding the transected nerve end at 0 hours and 24 hours were evaluated using an unpaired *t* test; differences between the quantity of mRNA in the transected side of ganglion tissue and in the naïve side were examined using one-way ANOVA and post hoc analysis was performed using Tukey's method. $P < .05$ was considered statistically significant.

Results

Changes in BDNF mRNA Expression

BDNF mRNA was measured by real-time PCR. Each PCR product was confirmed to be a single band (data not shown). In the transected nerve and surrounding tissues, BDNF mRNA (expressed as the ratio of BDNF to β-actin) was 0.265 ± 0.038 at 0 hours (pretransection) and 0.807 ± 0.14 at 24 hours after transection (unpaired *t* test, $P < .01$). In the trigeminal ganglion (Fig 1) on the transected side, the level of BDNF mRNA was 0.495 ± 0.091 at 0 hours and 1.592 ± 0.638 at 24 hours after transection; this was a statistically significant difference (one-way ANOVA, $P < .01$). At 24 hours after transection, significantly higher BDNF mRNA expression was detected on the transected side compared with the naïve (nontransected contralateral) side (naïve side: 0.699 ± 0.278, one-way ANOVA, $P < .05$). There was no significant difference in BDNF mRNA expression between 0 hours and 24 hours on the naïve side, or between the transected and naïve side at 0 hours (one-way ANOVA, $P > .05$).

Histopathologic Observations

Photographs of the transected region 3 weeks after nerve transection in each group are presented in Fig 2. Figure 2a shows the change in the NS group. Nerve regeneration was evident in the A-B1 group (Fig 2b). Compared with the A-B1 group, the NS group exhibited neuroma formation with medial to distal swelling; the regenerated nerve tissue in the transected nerve region in this group was rich in collagen fibers, but there was no apparent deformity of regenerated nerve trunks as was observed in the A-B1 group. In the A-B10 group (Fig 2c), the transected nerve did not show regeneration or connection. Furthermore, no involvement of collagen tissue was evident in the transected end of the nerve. Conversely, in the LN group, swelling of the nerve trunks was observed medially and distally to the transected nerve ends, and the axons in this lesion did not appear to be reconnected (Fig 2d).

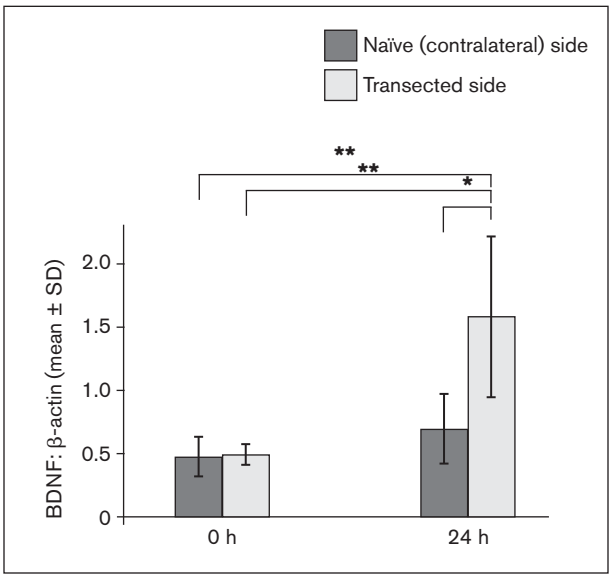

Fig 1 Quantitative differences in BDNF mRNA expression in the trigeminal ganglion at 0 hours and 24 hours after IAN transection. Levels of BDNF mRNA were significantly greater 24 hours after transection than before transection and also significantly greater in the transected side than in the naïve (nontransected contralateral) side. Relative BDNF expression is represented by the BDNF: β-actin ratio. Bars represent means ± SD (n = 3 per sample). *$P < .05$; **$P < .01$; one-way ANOVA.

Head Withdrawal Threshold

Chronologic changes in the head withdrawal threshold in response to mechanical stimuli are shown in Fig 3. In the transected side of all groups (Fig 3a), the head withdrawal threshold was elevated just after the transection and reached a maximum level 3 days after transection, with no significant differences between the groups on this day. Thresholds in all groups then decreased for the duration of the experiment (Fig 3a). There was no change in head withdrawal threshold in the naïve (nontransected contralateral) side in any group (Fig 3b). In contrast, the LN group exhibited no decrease in threshold at day 3, and this level remained above the cut-off level throughout the 3 weeks (data not shown; no statistical analysis against other groups was performed).

Head Withdrawal Threshold Changes Within Each Group

In all groups, 3 days after transection, the mean head withdrawal thresholds rose to 58 g (almost corresponding to the cut-off level).

In the NS group, the thresholds on days 14 and 21 after transection were significantly lower than the maximal level observed on day 3 (two-way repeated measures ANOVA, both $P < .001$). In the A-B1 group, the threshold decreased after day 3, resulting in thresholds on days 14 and 21 that approximated

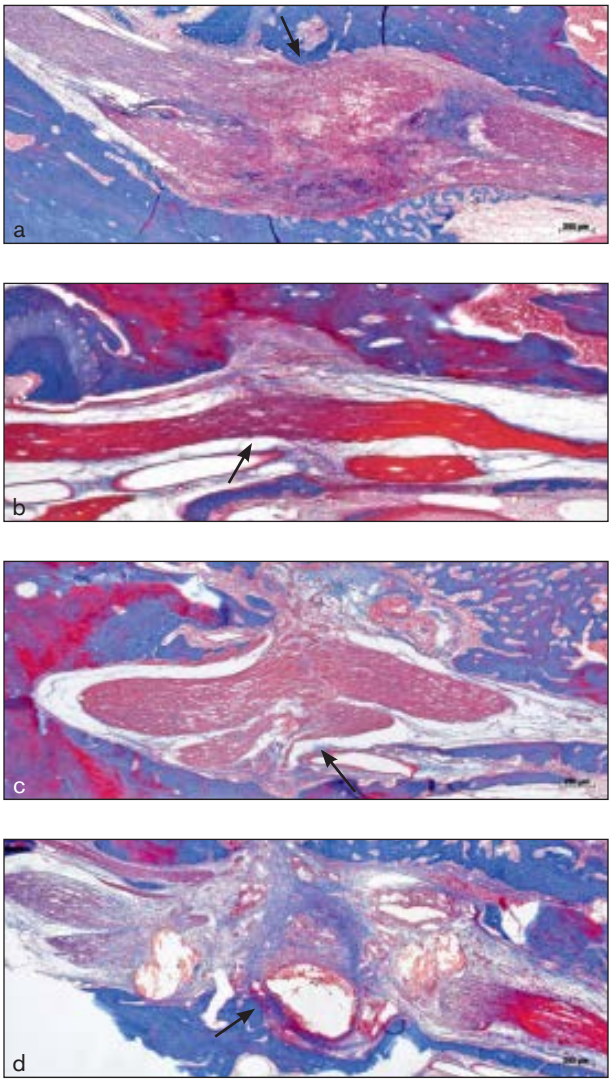

Fig 2 Morphologic analysis of the regenerated IAN 3 weeks after transection; Azan staining. **(a)** NS group; **(b)** A-B1 group; **(c)** A-B10 group; **(d)** LN group. The right and left sides indicate the central and peripheral sides of the IAN, respectively. Arrows indicate the site of transection of the IAN. Scale bars indicate 200 μm. Neuroma developed in the NS group **(a).** In the A-B1 group **(b)**, there were no notably enlarged tissues at the site of nerve regeneration; administration of 1 μg BDNF antibody inhibited neuroma formation. In the A-B10 group **(c),** 10 μg BDNF antibody inhibited nerve regeneration. Moreover, in the animals in the LN group **(d),** in which the transected nerve ends were tightly ligated immediately after transection, sprouting from the transection site and regeneration were completely inhibited.

the same level as observed before transection; the head withdrawal threshold at day 21 was 20.6 ± 6.8 g, which was not significantly different from the pretransection value (28.3 ± 5.7 g). No significant changes in the threshold were discernible among days 0, 14, and 21 in the A-B1 group (two-way repeated measures ANOVA, $P > .05$).

The A-B10 group exhibited a decrease in head withdrawal threshold after day 3, but the thresholds on days 7 and 14 were significantly higher than the pretransection thresholds (two-way repeated measures ANOVA, $P < .001$). The threshold on day 21 was 27.1 ± 12.6 g compared with 19.1 ± 7.7 g before transection, but this difference was not significant.

Comparisons Among NS, A-B1, and A-B10 Groups

At day 7, head withdrawal thresholds on the transected side were 24.5 ± 6.3 g (NS), 36.7 ± 12.4 g (A-B1), and 51 ± 10.7 g (A-B10). On day 14, they were 7.3 ± 2.1 g (NS), 24.1 ± 6.8 g (A-B1), and 38.3 ± 9.4 g (A-B10). Head withdrawal thresholds were significantly higher in the A-B1 and A-B10 groups than in the NS group (two-way repeated measures ANOVA, both $P < .01$) at both time points (two-way repeated measures ANOVA, both $P < .01$). However, the threshold of the A-B10 group was significantly higher than that of the A-B1 group (two-way repeated measures ANOVA, $P < .01$).

At day 21, the threshold of the NS group decreased to 6.7 ± 2.4 g, which was the lowest overall threshold observed among all three groups for the 3 weeks of measurements (two-way repeated measures ANOVA, all $P < .01$). No significant difference was observed between the A-B1 and A-B10 groups at day 21 (two-way repeated measures ANOVA, $P > .05$).

In contrast, the head withdrawal thresholds on the naïve side did not show any significant change throughout the entire observation period, and no significant differences were found in the naïve thresholds across all groups (Fig 3b; two-way repeated measures ANOVA).

JOR

The JOR was evoked in the anterior belly of the digastric muscle on the transected side in the NS (Fig 4a) and A-B1 (Fig 4b) groups. In contrast, the LN group did not exhibit any JOR (Fig 4c). The thresholds did not differ significantly between the A-B1 and NS groups when the mental nerve was stimulated on the same side (Table 1; unpaired *t* test, $P > .05$).

A comparison of the latency between the A-B1 and NS groups revealed no statistically significant difference (Table 1; unpaired *t* test, $P > .05$).

Discussion

This study has shown that local administration of an antibody against BDNF at a trigeminal nerve injury site modulates regeneration of the injured nerve. Furthermore, the dose administered is an important factor in the recovery of the sensorimotor response

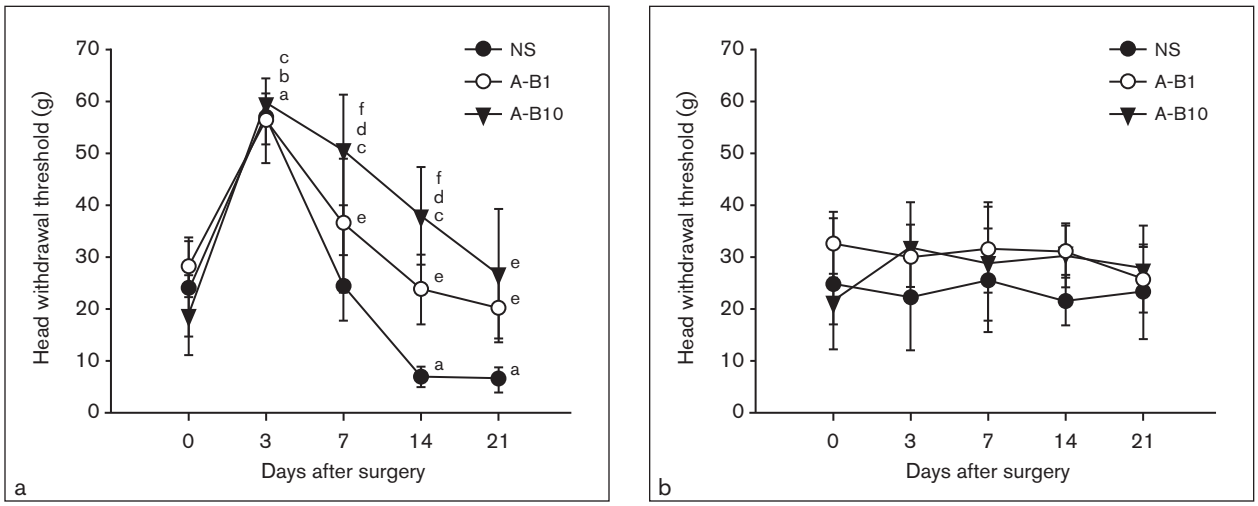

Fig 3 Changes in head withdrawal threshold in response to mechanical stimulation. Data are the mean ± SD (g). **(a)** Head withdrawal threshold evoked by mechanical stimulation of the mental region on the transected side. In the NS group, the threshold continued to decrease, and 3 weeks after transection it was lower than before transection. In the A-B1 group, 3 weeks after transection, the threshold had almost returned to the same level as that prior to transection. In the A-B10 group, 3 weeks after transection, the threshold had not returned to the same level as before transection. **(b)** Head withdrawal threshold evoked by mechanical stimulation of the mental region on the naïve (nontransected contralateral) side. There were no significant changes during the observation period in any group. [a]$P < .001$ vs 0 days in the NS group; [b]$P < .001$ vs 0 days in the A-B1 group; [c]$P < .001$ vs 0 days in the A-B10 group; [d]$P < .001$, [e]$P < .01$ vs each day in the NS group; [f]$P < .01$ vs each day in the A-B1 and A-B10 groups (two-way repeated measures ANOVA). Data represent the mean ± SD (n = 6).

Table 1 Threshold and Latency of the JOR Evoked by Electrical Stimulation of the Transected Side of the IAN in the Mental Region (Mean ± SD)

Groups	Threshold (μA)	Latency (ms)
NS (n = 6)	371.6 ± 154.8	6.26 ± 0.773
AB-1 (n = 6)	375 ± 330.2	6.66 ± 0.993
P	.983	.456

to mechanical stimulation in the area supplied by the injured nerve. The higher dose of BDNF antibody inhibited sensorimotor recovery, resulting in an increased head withdrawal threshold to mechanical stimulation. In contrast, the lower dose resulted in a decreased head withdrawal threshold. As BDNF facilitates neurite growth and elongation,[19] it can be considered that control of locally induced BDNF affects the reconnection of a transected peripheral nerve. Furthermore, BDNF may also be involved in nocifensive mechanisms after nerve injury. This study demonstrates that endogenous BDNF at a trigeminal nerve injury site has a critical functional and morphologic role in the regenerative process of the injured nerve.

Injury Induces Local BDNF Production and TrkB Expression

Sciatic nerve ligation induces immunoexpression of BDNF at the proximal and distal sides of the nerve injury and in the soma of dorsal root ganglion (DRG)

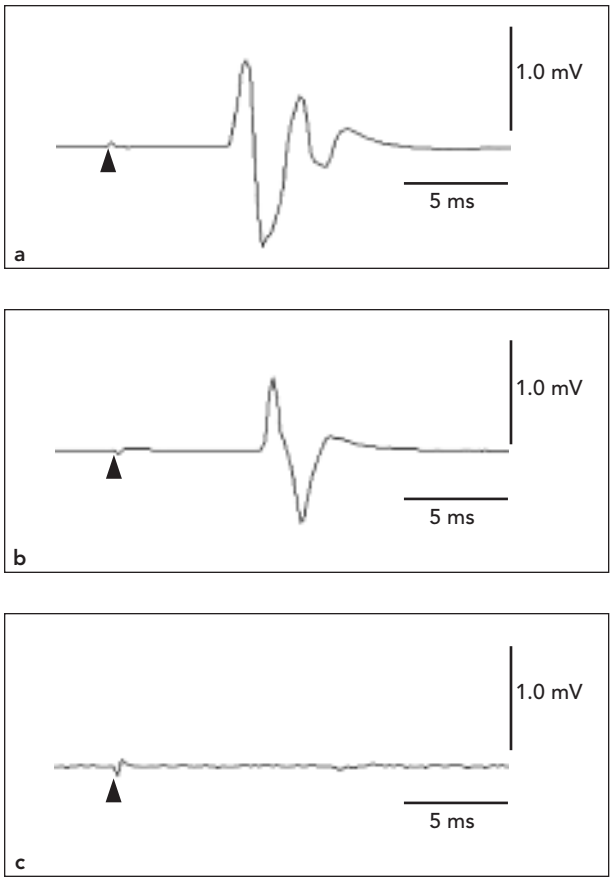

Fig 4 Examples of the jaw-opening reflex (JOR) evoked by electrical stimulation of the transected IAN. The JOR was evoked in the NS group **(a)** and A-B1 group **(b),** but not in the LN group **(c).** Arrowheads indicate stimulation points.

neurons and central terminals in lamina II of the spinal dorsal horn. These findings suggest that nerve injury triggers BDNF production, but from two different origins: BDNF produced in the sensory neuron ganglia, which is transported to central terminals, and BDNF detected in the distal end of the nerve that is not derived from the neuron.[20] Axotomy can induce upregulation of neurotrophins in non-neuronal cells within 1 day of injury[21] and nerve injury upregulates BDNF synthesis in the non-neuronal cells of the nerve sheath.[22] Neurotrophin receptors mRNA, including p75, TrkB, and TrkC, increase in expression 1 week after axotomy, but TrkB mRNA expression is particularly increased at the proximal end of the nerve.[23] These findings suggest that locally existing BDNF at the injury site acts on TrkB, leading to regeneration of the injured axons.

Sites of Action of Administered BDNF Antibody

The capacity of axotomized motoneurons to regenerate their axons depends on the time from injury and response to chronologic changes in BDNF after nerve injury.[24,25] The expression of BDNF in the DRG increases as early as 24 hours after spinal nerve injury and this elevated expression lasts for at least 2 weeks.[26,27] This is consistent with the present findings in the trigeminal ganglion. In contrast, axotomy initiates a slow increase of BDNF mRNA that starts 3 days postlesion and reaches maximal levels 3 to 4 weeks later. Furthermore, the early increase in BDNF can be attributed to Schwann cells, which are a rich source of BDNF.[22,28]

Funakoshi et al[23] reported that BDNF mRNA expression increases much later, 1 week after sciatic nerve injury, whereas the expression in the spinal cord decreases after axotomy. TrkB mRNA expression increases 6 hours after axotomy in the proximal end of the axotomized nerve but decreases to pre-axotomy levels 3 days later. The TrkB receptor was detectable in the part of the nerve proximal to the lesion, in contrast to p75 receptor expression, which is found in the distal site.[23] In the present study, it was not possible to detect any changes in BDNF mRNA from the nerve tissue alone (data not shown). However, significant change occurred in the tissue surrounding the nerve lesion, suggesting that the changes in BDNF mRNA are derived from perineurium and/or connective tissue around the nerve lesion. A previous study reported that endogenous BDNF is transported from the DRG neurons to the proximal end of the nerve and secreted along the axonal surface in an anterograde fashion.[29] Fibroblasts and Schwann cells release BDNF-like molecules.[30] Taken together, it can be speculated that, immediately after axotomy of a trigeminal nerve, an exogenously administered antibody to BDNF can affect local BDNF derived from the trigeminal ganglion and released from the proximal nerve stumps, and that this BDNF is not derived from either Schwann cells or fibroblasts in the vicinity of the lesion.

BDNF is Involved in Nerve Injury–Induced Changes in Sensorimotor Function

Immediate local administration of BDNF has no facilitative effect in the regeneration of the lingual nerve after resection.[31] Because neutralization of endogenous BDNF by an antibody reduces neurite outgrowth of sensory neurons, it also impairs the ability of injured neurons to extend neurites.[11] Peripheral axotomy induces some changes in sensory function during the regeneration period. It downregulates the expression of BDNF in small neurons and switches to large neurons, meaning that the change in BDNF phenotype may relate to neuropathic pain.[26] Tsuboi et al[14] demonstrated that the regenerated IAN contains many large unmyelinated axons and thin myelinated axons distal to the transected site. These changes in the features of regenerated fibers and/or newly expressed channels[32–34] in a regenerated nerve may relate to the change in sensitivity of the regenerated nerve.[14] When local BDNF is inactivated, neuropathic behavior in the form of autotomy is markedly reduced after nerve transection.[35] Analyses of the JOR in the present study demonstrated regeneration of the damaged nerve, but could not address the effect of the antibody on the changes in fiber content of the regenerated nerve. This is because the short distance between the stimulation site and recording site made it difficult to detect changes in latency that might be attributed to differential effects on slowly and rapidly conducting afferent fibers. Further studies will be required to address this.

Retrograde labeling of motoneurons has been used to reveal two phases in the dose-dependent effects of motor function regenerated by local administration of BDNF to the injury site.[1] Low doses of BDNF did not induce a detectable effect on axonal regeneration; conversely, a high dose of BDNF inhibited regeneration. The present study used a BDNF antibody to control local BDNF at the lesion site. This meant that local administration of saline had no neutralizing effect on local BDNF, which corresponded to a higher activity of BDNF, resulting in a decreased head withdrawal threshold and greater sensitivity. In contrast, local administration of a high dose of BDNF antibody produced a decrease in activity of local BDNF, resulting in higher thresholds and less sensitivity, which is suggestive of poor regeneration. These results indicate that the local BDNF facilitated sensory nerve regeneration, resulting in excessive elongation of the regenerating nerve. These effects caused mechanical threshold changes and suggest

a similar phenomenon to that observed by Boyd and Gordon.[1] Therefore, immediate control of local BDNF can determine the prognosis of injured sensory nerve function.

Central Contribution of BDNF to Nerve Injury–Induced Changes in Sensorimotor Function

In peripheral nerves, sodium channels accumulate at the end of transected nerves and this can account for hyperexcitability and consequent neuropathic pain.[36,37] Neurotrophins such as nerve growth factor, glial cell-derived neurotrophic factor, and BDNF activate mitogen-activated protein kinases and these can promote expression of sodium channels in the primary neuron.[38]

To consider the mechanisms involved in the change of sensorimotor thresholds after nerve injury, central effects must be explained in addition to peripheral action. BDNF infusion to the brain and the spinal cord decreased paw flinch response in the formalin test.[39] Intrathecal application of an antibody to BDNF attenuates thermal hyperalgesia after spinal nerve ligation.[10] BDNF also increases the frequency of excitatory postsynaptic currents in animals with inflammation, and this increase is associated with sodium channels on TrkB receptors.[40] BDNF can also transfer information between activated microglia and neurons during central sensitization.[41] Therefore, these central mechanisms caused by peripheral nociceptive inputs could also explain the changes in sensorimotor responses during recovery from peripheral nerve injury.

BDNF-Mediated Traumatic Neuroma Formation

When BDNF binds to the TrkB receptor in proximal nerve stumps, intracellular cyclic adenosine monophosphate (cAMP) is activated, which leads to the initiation of gene transcription and translation and results in axon outgrowth.[42] TrkB receptor-deficient mice exhibit neuroma formation after axotomy; in contrast, wild-type animals receiving a nerve graft derived from the normal or knockout mouse do not exhibit neuroma formation, suggesting that TrkB has an important role in neuroma formation.[9] Local administration of a BDNF antibody at the injury site decreases the incidence of neuroma formation, but does not influence neuron regeneration,[16] which is consistent with previous findings that locally neutralizing BDNF does not inhibit reconnection of the resected axon.[17] Therefore, these results suggest that BDNF triggered by nerve injury acts on TrkB receptors in the proximal nerve stump and induces proliferative outgrowth of the axon, resulting in neuroma formation.

Clinical Implications

Patients with IAN injury suffer from long-term changes in sensation in the mental region and sometimes prolonged severe pain and/or dysesthesia. Therefore, following injury, clinical observations of mechanical (tactile) thresholds in the mental region, especially persistent lowered thresholds, can be a good indicator of possible neuroma formation at an IAN injury site. Early treatment to control local BDNF levels within a few days of injury is desirable to prevent excessive sprouting of the regenerating nerve and excessive stimulation of local and central pain mechanisms.

In conclusion, IAN damage causes production of BDNF in the trigeminal ganglion and the nerve at the IAN transection site. BDNF may promote axonal regeneration and affect recovery of sensorimotor responses, resulting in either hyper- or hyposensitivity depending on the amount of local BDNF.

Author Contributions

H.Y and K.S. designed the experiments. H.Y. performed all experiments and drafted the manuscript. Y.Y. and T.M. carried out the histopathologic analysis. M.K. and K.Y carried out the JOR experiments and analyzed the data with H.Y. The study was conceived by K.S., who also contributed to its design and coordination and helped draft the manuscript. All authors read and approved the final manuscript.

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