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The early, long-term inhibition of brain-derived neurotrophic factor improves voiding, and storage dysfunctions in mice with spinal cord injury

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Abstract

Aims: We examined the time course of urodynamic changes and the effect of the short or long-term inhibition of brain-derived neurotrophic factor (BDNF) from the early phase after spinal cord injury (SCI) in mice.

Methods: The spinal cord of female C57BL/6N mice was completely transected. We examined filling cystometry and bladder BDNF levels at 10, 20, and 30 days after SCI, with an additional day-5 measurement of BDNF. In a separate group of mice, anti-BDNF antibody (Ab) (10 μg/kg/h) was subcutaneously administered using osmotic pumps from day 3 after SCI, and single-filling cystometry was performed at 10 and 30 days (7 and 27 days of treatment, respectively) after SCI.

Results: Compared to spinal intact mice, bladder mucosal BDNF was increased at each time point after SCI with the maximal level at day 5 after SCI. Voiding efficiency was lower at each time point after SCI than that of spinal intact mice. The number of non-voiding contractions (NVC) during bladder filling was gradually increased with time. In both 10- and 30-day SCI groups treated with anti-BDNF Ab, voiding efficiency was improved, and the duration of notch-like intravesical pressure reductions during voiding bladder contractions was prolonged. The number of NVC was significantly decreased only in 30-day SCI mice with 27-day anti-BDNF treatment.

Conclusions: Overexpression of BDNF is associated with the deterioration of voiding efficiency after SCI. The early-started, long-term inhibition of BDNF improved voiding dysfunction and was also effective to reduce the later-phase development of detrusor overactivity after SCI.

KEYWORDS

brain-derived neurotropic factor, detrusor sphincter dyssynergia, spinal cord injury

1 | INTRODUCTION

Spinal diseases including spinal cord injury (SCI) induce neurogenic bladder and urethral dysfunction such as detrusor overactivity (DO) and detrusor-sphincter dyssynergia (DSD).[1] In SCI, the association of DO and dyssynergic activity of the external urethral sphincter (EUS) increases the risk of renal dysfunction due to high

intravesical pressure during bladder contractions as well as voiding inefficiency with high residual volume. The neural mechanisms of DO and DSD have been studied mainly using rat models of SCI.[2]

In SCI rats, neurotrophic factors including brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) reportedly contribute to the emergence of non-voiding contractions (NVC) shown by uninhibited bladder contractions without voiding in urodynamic testing. Increased NGF levels in the bladder and the spinal cord were associated with NVC in SCI rats, and the administration of anti-NGF antibody in the spinal cord inhibited the emergence of NVC almost completely.[3,4] According to recent literature, BDNF is also related to the development and maintenance of NVC in SCI rats.[5] Therefore, it seems that neurotrophic factors such as NGF and BDNF play important roles in bladder storage dysfunction in SCI rats.

In a recent study using cystometry and EUS-electromyogram (EMG) recordings, the late-phase, systemic administration of anti-BDNF antibody for 7 days ameliorated DSD with the improvement of voiding efficiency, but did not reduce NVC in 4-weeks SCI mice.[6] According to these previous findings, BDNF could play different roles in the SCI-induced lower urinary tract dysfunction between rats and mice.[5,6] Also, there are several differences between SCI rats and mice regarding the coordination of bladder and EUS activity during voiding. In SCI rats, voiding efficiency recovers with time due to the re-emergence of EUS pumping activity during voiding, which is absent in humans, whereas severe inefficient voiding continues in SCI mice.[7] Therefore, it seems that SCI mice are more appropriate for studying the role of BDNF in SCI-induced voiding dysfunction compared to SCI rats whose voiding efficiency recovers substantially without treatment. Furthermore, a previous study examined the effect of short-term, late-phase administration of anti-BDNF antibody in SCI mice[6]; however, it is not known whether early-started BDNF inhibition has any effect on lower urinary tract dysfunction in SCI mice. Thus, we investigated the effect of short- or long-term inhibition of BDNF that was started from the early phase of SCI on lower urinary tract function in SCI mice after exploring the time-dependent urodynamic changes after SCI.

2 | MATERIALS AND METHODS

All procedures were conducted according to the NIH guidelines, and approved by the Institutional Animal Care and Use Committee.

2.1 | Animals and antibody

We used 8-week-old female C57BL/6N mice (body weight, 18-22 g), and completely transected the T8-9 spinal cord to produce the SCI model. After spinal cord transection, we manually squeezed their bladders to prevent urinary retention once daily every day until cystometric evaluation, as previously reported.[6] Spinal intact mice without surgery (n = 6) were also used for cystometric evaluation and measuring BDNF protein levels in the bladder. First, we studied the time-course changes of urodynamic parameters in SCI mice.

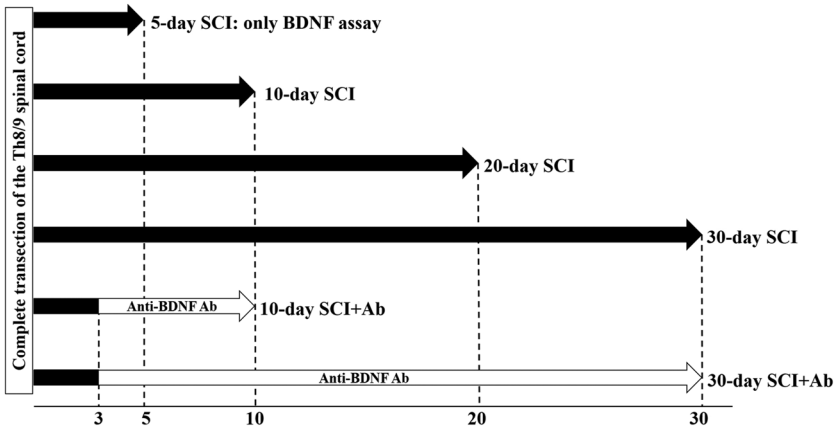


FIGURE 1 Experimental design. Cystometric evaluation and bladder BDNF measurements were performed at 10, 20, and 30 days after SCI (n = 8 in each) with additional BDNF assay at 5 days after SCI (n = 5). In some SCI animals, anti-BDNF Ab was continuously administered from 3 days after SCI, and cystometric evaluation and bladder BDNF measurements were performed at 10 and 30 days after SCI (n = 8 in each). In other words, anti-BDNF Ab was continuously administered to SCI mice for 7 or 27 days, respectively, until the evaluation. BDNF, brain-derived neurotrophic factor; SCI, spinal cord injury

As shown in the experimental plan (Figure 1), we performed cystometry (CMG) and measured bladder BDNF protein levels at 10, 20, and 30 days after spinal transection (n = 8 at each time point). BDNF levels were also measured at 5 days after spinal transection (n = 5) to check the early-point expression. In a separate group of animals, we examined the early and long-term effects of BDNF inhibition on urodynamic parameters in SCI mice. For BDNF inhibition, at 3 days after spinal transection, an osmotic pump containing anti-BDNF Ab (10 μg/kg/h) (R&D Systems, Inc, Minneapolis, MN) was placed subcutaneously in the back, and CMG and bladder BDNF protein levels were monitored at 10 and 30 days after spinal transection (n = 8 at each time point). In other words, anti-BDNF Ab was continuously administered to SCI mice for 7 or 27 days until the evaluation, respectively. The urodynamic change after anti-BDNF Ab was not evaluated in 20-day SCI mice because the urodynamic characteristics between 20- and 30-day SCI mice were similar, as described in Section 3 (Figure 2).

2.2 | Cystometric measurements

As previously reported,[6,8] single-filling CMG was performed under an awake condition to evaluate bladder function. Animals were anesthetized using 1.5% to 2.0% isoflurane. After laparotomy, we inserted a PE-50 tube (Clay-Adams, Parsippany, NJ) with the end flared by an electrocauterizer into the bladder from the dome. After closing the abdominal wound, each mouse was gently restrained in a cage (Economy holder 15-30 g; Kent Scientific, Torrington, CT), and the catheter was connected via a T shape stopcock attached to a pressure transducer and a syringe pump. A local anesthetic, EMLA cream containing lidocaine 2.5% and prilocaine 2.5% was

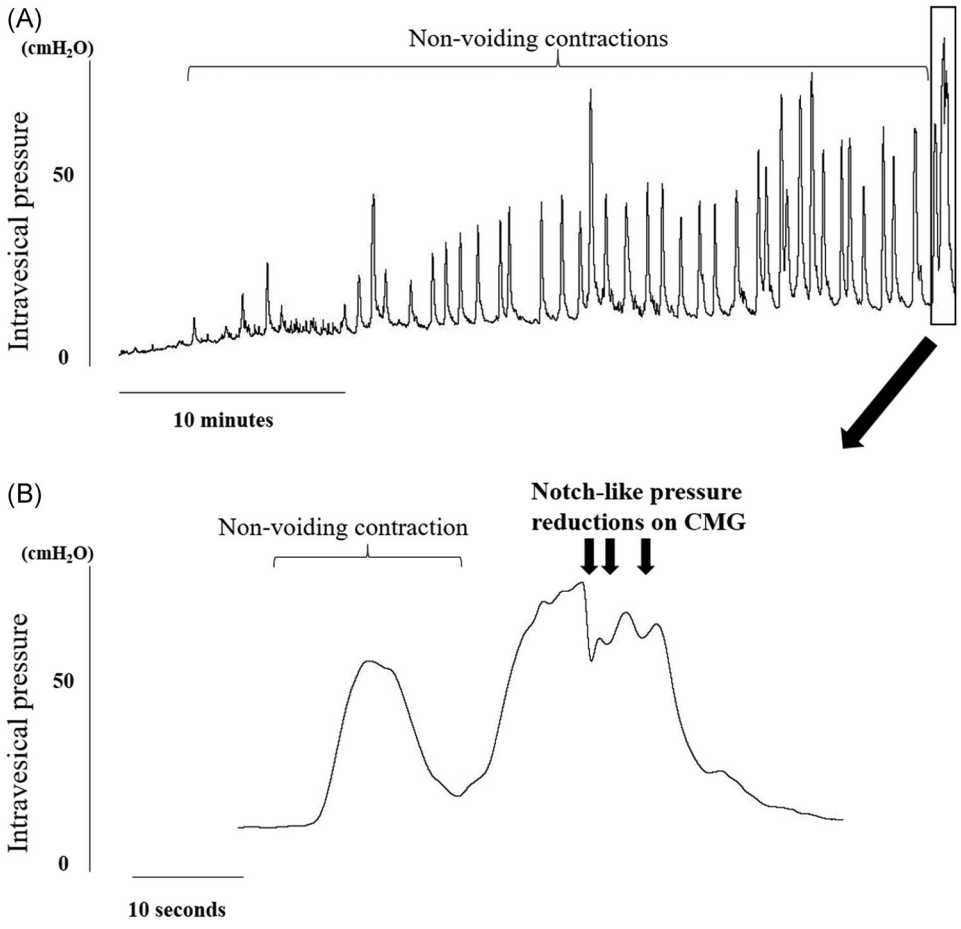


FIGURE 2 Representative cystometry (CMG) traces in a SCI mouse. A, An entire single-filling CMG trace showing storage and voiding phases of micturition. B, A CMG trace with expanded time scale, which is a portion of Trace A indicated by a rectangular box, showing intermittent voiding coincided with notch-like reductions in intravesical pressure. According to previous reports, actual voids, and the reduced EMG-EUS activity occur during these notch-like reduction periods.[6,7] EMG-EUS, electromyogram-external urethral sphincter; SCI, spinal cord injury

applied onto the abdominal skin wound to reduce surgical pain during CMG recordings. After complete recovery from anesthesia, we performed CMG with intravesical saline (0.01 mL/min) infusion for at least 90 minutes, to monitor stable bladder contractions. After stabilization of CMG traces and emptying the bladder through the catheter, the evaluation of single-filling CMG was immediately started by infusing saline. CMG parameters included: (a) the number of non-voiding contractions (NVCs), defined as transient bladder contractions with intravesical pressure greater than 8 cm H_2O above the baseline, without fluid release from the external urethral orifice, during bladder filling, (b) threshold pressure (TP), which was defined as intravesical pressure at the initiation of bladder contraction inducing voiding, (c) maximal voiding pressure (MP) and (d) post-void residual (PVR) withdrawn through the intravesical catheter by gravity after the end of micturition. Voiding events were observed as the release of liquid from the urethral meatus. Bladder capacity and voided volume (VV) were calculated by the following equation; bladder capacity = infusion rate (0.01 mL/min) × the time to void (minutes) after starting the saline infusion, and VV was calculated by subtraction of measured PVR from bladder capacity because VV was too little to be measured directly. Voiding efficiency (VV/bladder capacity × 100) was also calculated. These parameters were evaluated by using Chart software from AD Instruments (Colorado Springs, CO). Single-filling CMG recordings were performed twice during two micturition cycles with bladder emptying before starting each cycle in each animal to obtain the averaged value of CMG parameters for statistical analyses.

Previous studies using simultaneous CMG and EUS-EMG recordings[6,7] showed that notch-like reductions of intravesical pressure on CMG traces during voiding contraction coincide with the timing of actual voids as well as reductions in EUS-EMG activities periodically seen during tonic EMG firings. Therefore, we evaluated these notch-like periods on CMG traces to evaluate the degree of synergistic activity between the bladder and the EUS, by measuring the total time of notch-like reduction periods and its ratio to the bladder contraction time on CMG traces.

2.3 | Protein assay

After CMG evaluations, the animals were killed, and their bladders were promptly harvested. The bladder was opened and divided into bladder mucosa and muscle layers using microscissors under a microscope. Each tissue was preserved at −80°C until the protein assay. BDNF Emax ImmunoAssay Systems from Promega Co, Ltd (Madison, WI) were used to measure the bladder mucosal and muscle BDNF protein concentrations in accordance with the manufacture's instruction. The assayed BDNF values were standardized with the tissue protein concentrations (μg/mg protein) measured by the BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA).

2.4 | Statistics analysis

Results were presented as mean ± SD. Student *t*-test was used for statistical comparison of urodynamic results between two groups. One-way ANOVA was also used to compare the parameters among the three groups, followed by multiple comparisons with Bonferroni correction. $P < .05$ was considered to indicate statistical significance.

3 | RESULTS

3.1 | Time course changes after SCI

3.1.1 | Cystometric analysis

Compared to spinal intact mice, the number of NVC per void and threshold pressure was gradually and significantly increased with time after SCI (Figures 3 and 4). The number of NVC of 30-day SCI mice was significantly larger than that of 10-day SCI mice (37.3 ± 18.7 vs 10.5 ± 4.8 per void, $P < .05$). Voiding pressure among any SCI group was similar and significantly larger than that of spinal intact mice. The voided volume of 10-day SCI mice was smaller than that of spinal intact mice (0.018 ± 0.014 vs 0.064 ± 0.018 mL; $P < .05$). PVR and bladder capacity were larger in all three groups of SCI mice than those of spinal intact mice, and were larger in 20- and 30-day SCI mice than those of 10-day SCI mice. Voiding efficiency among the three SCI groups was similar but significantly smaller than that of spinal intact mice. Any of the significant differences did not include zero of 95% confidential intervals (CI) (Supporting Information Table).

3.2 | BDNF protein assay in the bladder

The bladder mucosal BDNF was significantly increased in all groups of SCI mice compared to spinal intact mice with the maximal peak at 5 days after SCI by 246% from 89 ± 35 to 309 ± 106 pg/mg protein (95% CI of difference, −∞

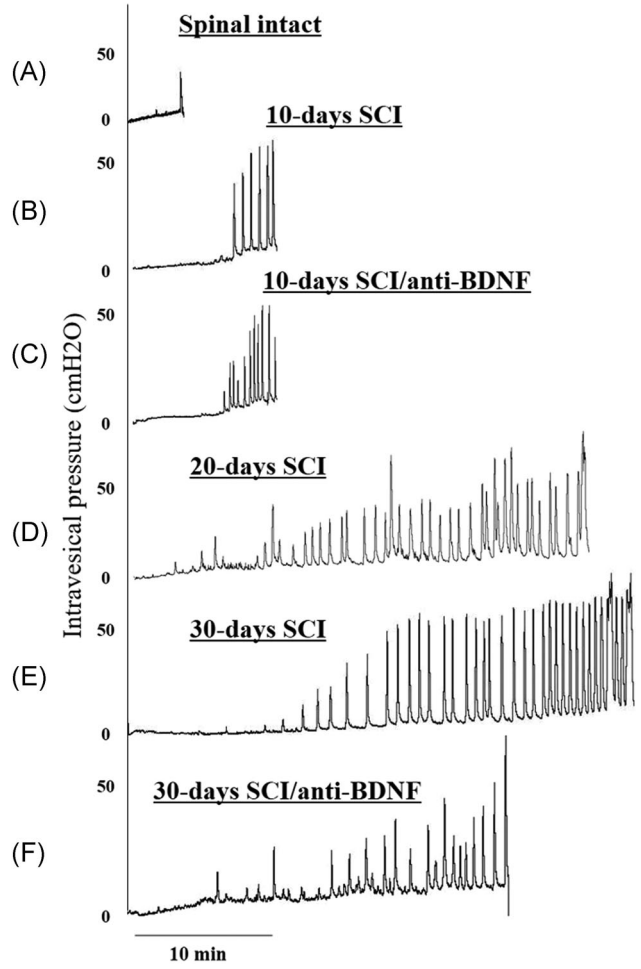


FIGURE 3 Representative CMG traces in spinal intact and SCI mice without or with anti-BDNF antibody (Ab) treatment. A, Spinal intact. B, Ten-day SCI. C, Ten-day SCI and anti-BDNF treatment. D, Twenty-day SCI. E, Thirty-day SCI. F, Thirty-day SCI and anti-BDNF treatment. The gradual increase of non-voiding contractions (NVC) with time at 10, 20, and 30 days was observed after SCI. The early-started, short-term (7 days) administration of anti-BDNF Ab did not significantly affect NVC, while the early-started, long-term (27 days) administration of anti-BDNF Ab significantly decreased NVC. BDNF, brain-derived neurotrophic factor; CMG, cystometry; SCI, spinal cord injury

to −136: $P < .05$; Figure 5). In the bladder muscle layer, BDNF concentrations were similar among SCI and spinal intact groups.

3.3 | Effects of early-started, short, or long-term BDNF neutralization

3.3.1 | Cystometric analysis

In 10-day SCI mice with 7-day of BDNF inhibition voiding efficiency was elevated from 14.7% ± 10.1% to 32.2% ± 16.6% (95% CI of difference, −∞ to −5.1; Supporting Information Table) with increased voided volume (0.018 ± 0.014 to 0.076 ± 0.046 mL; 95% CI of difference, −∞ to −0.021), and the ratio of durations of notch-like intravesical pressure reductions to voiding bladder contraction time was prolonged by 49% from 3.9 ± 1.7 to 5.8 ± 2.5% (95% CI of difference, −∞ to 0.1) compared to untreated, 10-day SCI mice (Figure 6). In 30-day SCI mice with 27-day of BDNF inhibition, voiding efficiency was elevated from 15.9% ± 4.5% to 22.7% ± 10.3% (95% CI of difference, −∞ to −0.8) with decreased PVR (0.366 ± 0.167 to 0.261 ± 0.083 mL; 95% CI of difference, −0.019 to ∞), and the notch-like reduction duration to voiding contraction time was significantly prolonged by 141% from 1.7% ± 1.4% to 4.1% ± 1.3% (95% CI of difference, −∞ to −1.1) compared to untreated, 30-day SCI mice (Figure 6). In addition, the number of NVC was significantly decreased by 45% from 37.3 ± 18.7 to 20.6 ± 4.9 per void (95% CI of difference, 2.5 to ∞) which is in contrast to no significant reduction of NVC in SCI mice with short-term BDNF inhibition (Figures 3 and 6).

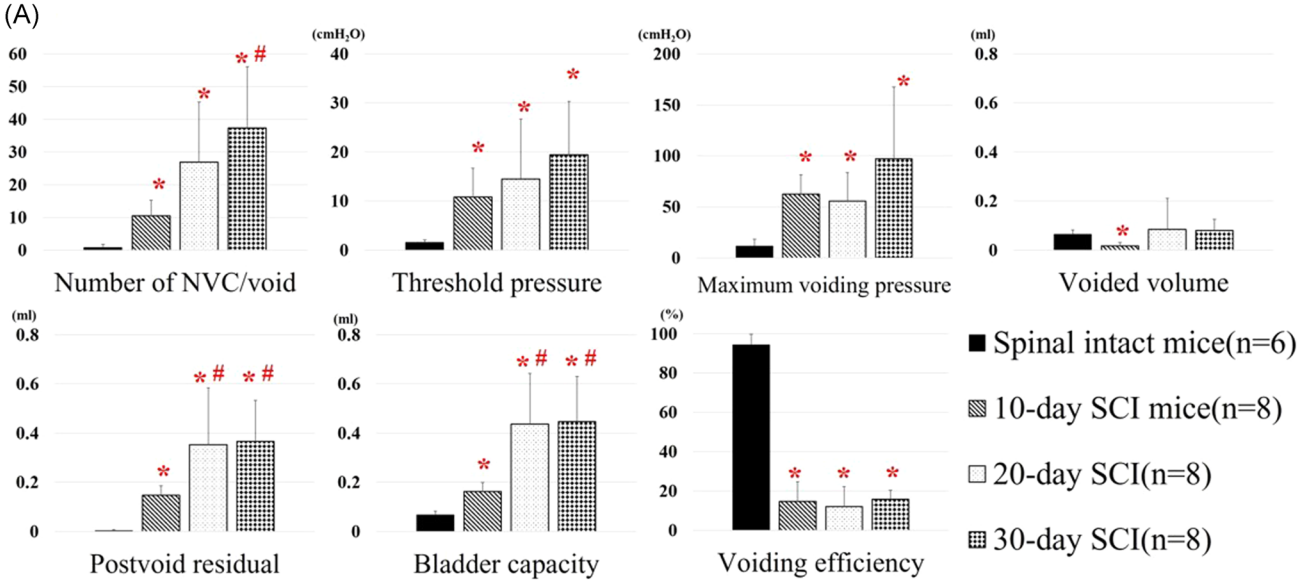
3.3.2 | BDNF protein assay in the bladder after BDNF inhibition (Figure 5)

BDNF concentrations in both bladder mucosa and muscle layers of both 10- (mucosa, 200 ± 67 to 60 ± 28 pg/mg protein; 95% CI of difference, 91 to ∞; muscle, 216 ± 91 to 116 ± 109 pg/mg protein; 95% CI of difference, 1.2 to ∞) and 30-day SCI mice (mucosa, 164 ± 36 to 81 ± 97 pg/mg protein; 95% CI of difference, 12 to ∞, muscle; 266 ± 154 to 113 ± 51 pg/mg protein; 95% CI of difference, 35 to ∞) was significantly decreased after anti-BDNF Ab treatment compared to untreated SCI mice.

4 | DISCUSSION

The findings of our study show that: (a) in SCI mice, voiding efficiency was equally very low at any time post-SCI while the incidence of NVC during bladder filling was gradually increased with time up to 30 days after SCI, (b) the early-started, short- and long-term inhibition of BDNF significantly improved voiding efficiency and prolonged the duration of notch-like intravesical pressure reductions during voiding bladder contraction, during which actual voids occurred, and (c) the early-started, long-term inhibition of BDNF was effective to reduce the later-phase development of DO after SCI. Thus, in SCI mice with inefficient voiding, BDNF plays a significant pathophysiological role in both storage and voiding dysfunctions after SCI, suggesting that BDNF-targeting treatments could be effective for treating lower urinary tract dysfunction in SCI.

(A)



(B)

	Number of NVC/void	Threshold pressure (cmH_2O)	Maximum voiding pressure (cmH_2O)	Voided volume (ml)	Postvoid Residual (ml)	Bladder capacity (ml)	Voiding efficiency (%)
Spinal intact mice (n=6)	0.8±1.0 (-1.4–3.5)	1.6±0.5 (0.0–3.2)	11.8±6.6 (-6.3–31.9)	0.064±0.018 (0.029–0.111)	0.003±0.005 (-0.001–0.003)	0.067±0.016 (0.030–0.113)	94.4±5.4 (95.3–101.7)
10-day SCI mice (n=8)	10.5±4.8* (5.8–17.4)	10.8±5.9* (5.6–18.0)	62.6±18.7* (43.3–86.1)	0.018±0.014* (-0.007–0.042)	0.148±0.038* (0.070–0.207)	0.162±0.037* (0.086–0.219)	14.7±10.1* (1.3–28.0)
20-day SCI mice (n=8)	26.9±18.3* (0.4–48.4)	14.5±12.2* (0.3–28.4)	55.6±27.9* (22.9–86.7)	0.085±0.126 (-0.046–0.237)	0.352±0.232*# (0.081–0.613)	0.437±0.205*# (0.207–0.677)	12.0±10.2* (-0.2–26.7)
30-day SCI mice (n=8)	37.3±18.7*# (16.1–59.1)	19.4±10.9* (6.8–32.0)	97.1±70.2* (36.4–171.4)	0.081±0.045 (0.033–0.112)	0.366±0.167*# (0.207–0.440)	0.447±0.184*# (0.281–0.510)	15.9±4.5* (10.5–20.8)

Mean ± SD, (95% CI) * P<0.05 versus spinal intact mice, # P<0.05 versus 10-day SCI mice

FIGURE 4 Analyses of urodynamic parameters in SCI mice. A, The number of NVC per void and threshold pressure were gradually and significantly increased with time after SCI. Voiding pressure among three SCI groups was similar and significantly larger than that of spinal intact mice. The voided volume of 10-day SCI mice was smaller than that of spinal intact mice, 20- and 30-day SCI mice. Post-void residual (PVR) and bladder capacity were larger in all groups of SCI mice than those of spinal intact mice, and were also larger in 20- and 30-day SCI mice than those of 10-day SCI mice. Voiding efficiency among three SCI groups was similar, but significantly smaller than that of spinal intact mice. *$P < .05$ vs spinal intact mice, #$P < .05$ vs 10-day SCI mice. B, Time-course changes in urodynamic parameters after SCI. NVC, non-voiding contractions; SCI, spinal cord injury

BDNF, one of the neurotrophins, which was isolated from the pig's brain as a neuronal survival-eliciting factor in the 1980s, is an important modulator of neural plasticity.[9] Over the last 30 years, the role of BDNF on SCI or other pathological conditions has been researched; however, there is still no uniformity with BDNF actions that mediate both adaptive and maladaptive plasticity.[10,11] Our observations indicated that overexpression of BDNF after SCI was associated with deleterious effects on lower urinary tract function because bladder overactivity and voiding inefficiency were improved by the inhibition of BDNF. BDNF induces various physiological and pathological modulation by acting on tropomyosin receptor kinase B (TrkB), which is the high affinity, ligand-specific receptor of BDNF and is present in many neurons in the spinal cord and primary afferent pathways.[11] A previous study also showed that anti-BDNF Ab reduced the protein level of BDNF that was overexpressed in the spinal cord as

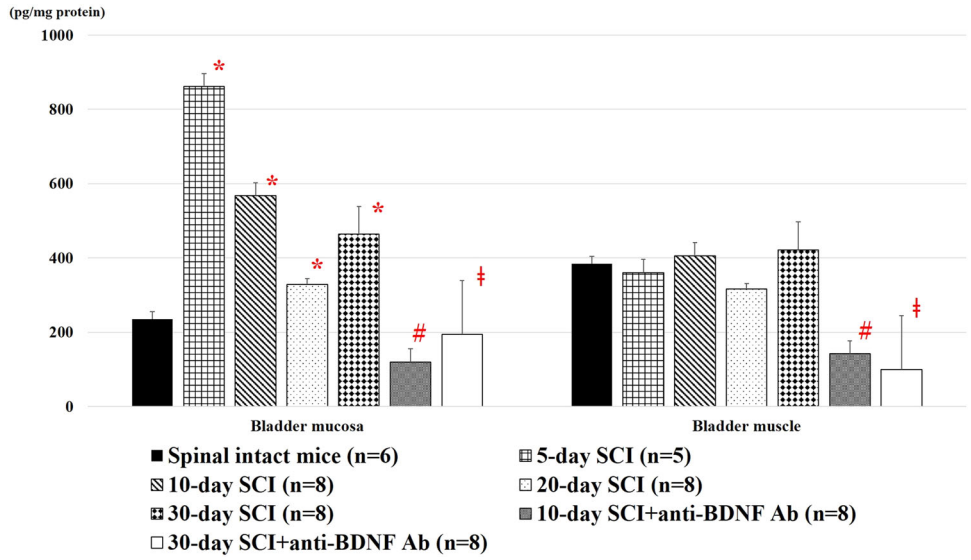


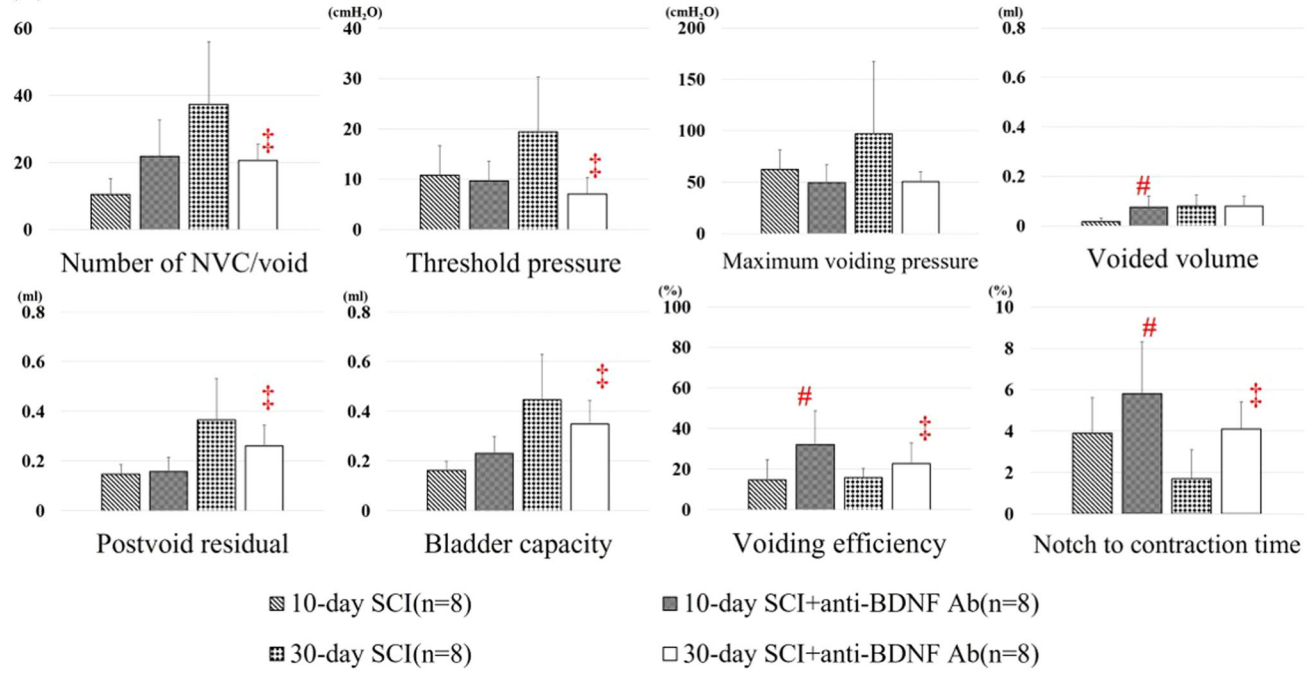
FIGURE 5 Bladder BDNF protein assay. The bladder mucosal BDNF was significantly increased in all groups of SCI mice compared to spinal intact mice with the maximal peak at 5-day after SCI while BDNF concentration in the detrusor was similar among spinal intact and SCI groups. BDNF concentrations in bladder mucosa and muscle layers of both 10- and 30-day SCI mice was significantly decreased after anti-BDNF Ab treatment. $^{*}P < .05$ vs spinal intact mice, $^{\#}P < .05$ vs 10-day SCI mice, $^{‡}P < .05$ vs 30-day SCI mice. BDNF, brain-derived neurotrophic factor; SCI, spinal cord injury

well as in the bladder of SCI mice.[6] While many reports suggest that increased BDNF could promote functional recovery and adaptive plasticity, the effects of BDNF on sensory functions might be maladaptive.[11] Previous studies showed increased messenger RNA (mRNA) levels of BDNF in the bladder after SCI [12] and the abundant production of BDNF in visceral epithelia including bladder urothelium.[13] Also, the urinary BDNF level of patients with a non-neurogenic overactive bladder is elevated, but is decreased in response to effective treatments.[14-16] Thus, the bladder urothelium, which is the main cellular component in the bladder mucosa, is likely to be a major source of BDNF production. In addition, it has been shown that BDNF protein levels are increased not only in the bladder, but also in the spinal cord [6] and that BDNF mRNA levels are altered in the spinal cord from SCI animals.[17] Therefore, another potential source of BDNF is the spinal cord, from which BDNF could be transported to the bladder mucosal region through afferent nerves although further studies are needed to clarify this point.

In this study, time-course changes in cystometric parameters showed the gradual increase of NVC with time after SCI although this gradual increase of NVC was not parallel with bladder BDNF expression levels after SCI. The short-term (7 days) inhibition of BDNF in 10-day SCI mice did not affect NVC although bladder BDNF expression was reduced. However, the early-started, long-term inhibition of BDNF for 27 days in 30-day SCI mice significantly decreased the number of NVC. This observation was consistent with the previous study showing that BDNF inhibition improved bladder function in chronic SCI rats whereas BDNF inhibition resulted in early development of bladder overactivity during the post-SCI spinal shock phase.[5] They also described that the premature sprouting of sensory afferents by BDNF inhibition induced early-phase neurogenic bladder overactivity, suggesting a protective role of BDNF in bladder function during the initial phase after SCI in rats.[5] Accordingly, our current results are likely to indicate that the early-started, short-term BDNF inhibition was not able to inhibit NVC, but rather contributed to the maintenance of NVC during the storage phase at 10 days after SCI.

In contrast to the short-term anti-BDNF treatment, the early-started, long-term anti-BDNF Ab administration decreased the number of NVC in chronic SCI mice at 30-day after spinal transection. In both SCI rats and mice, the afferent limb of micturition reflexes inducing NVC is likely to consist of C-fiber afferent pathways because capsaicin pretreatment, which induces desensitization of capsaicin-sensitive C-fiber afferent pathways, significantly reduced NVC in both species.[17,18] Thus, anti-BDNF Ab treatment significantly decreases the emergence of NVC probably due to the reduced BDNF action on C-fiber afferents. The receptor of BDNF, TrkB, is present on both large (Aδ-fiber) and small (C-fiber) cells in peripheral afferents although there seems to be more TrkB expression in large-sized, Aδ-fiber bladder afferent neurons.[19,20] Therefore, we speculated that the early-started, long-term inhibition of BDNF

(A)



(B)

	Number of NVC/void	Threshold pressure (cmH_2O)	Maximum voiding pressure (cmH_2O)	Voided volume (ml)	Postvoid residual (ml)	Bladder capacity (ml)	Voiding efficiency (%)	Notch to contraction time (%)
10-day SCI mice (n=8)	10.5±4.8 (5.8–17.4)	10.8±5.9 (5.6–18.0)	62.6±18.7 (43.3–86.1)	0.018±0.014 (-0.007–0.042)	0.148±0.038 (0.070–0.207)	0.162±0.037 (0.086–0.219)	14.7±10.1 (1.3–28.0)	3.9±1.7 (1.3–4.8)
10-day SCI mice +anti-BDNF Ab (n=8)	21.8±10.9 (14.0–33.2)	9.7±3.9 (5.6–8.0)	49.6±17.9 (43.3–86.1)	0.076±0.046 # (-0.007–0.042)	0.158±0.058 (0.070–0.207)	0.231±0.069 (0.086–0.219)	32.2±16.6 # (1.5–30.0)	5.8±2.5 # (1.5–5.0)
30-day SCI mice (n=8)	37.3±18.7 (16.1–59.1)	19.4±10.9 (6.8–32.0)	97.1±70.2 (36.4–171.4)	0.081±0.045 (0.033–0.112)	0.366±0.167 (0.207–0.440)	0.447±0.184 (0.281–0.510)	15.9±4.5 (10.5–20.8)	1.7±1.4 (0.4–3.1)
30-day SCI mice +anti-BDNF Ab (n=8)	20.6±4.9‡ (13.5–26.7)	7.1±3.3‡ (3.9–11.0)	50.7±9.5 (40.0–61.4)	0.080±0.040 (0.058–0.122)	0.261±0.083‡ (0.172–0.360)	0.349±0.095‡ (0.248–0.464)	22.7±10.3‡ (17.6–33.6)	4.1±1.3‡ (2.4–6.0)

Mean ± SD, (95% CI) # P<0.05 versus 10-day SCI mice, ‡ P<0.05 versus 30-day SCI mice

FIGURE 6 Analyses of urodynamic parameters in SCI mice with or without anti-BDNF treatment. A, In 10-day SCI mice with 7-day inhibition of BDNF, voiding efficiency was improved with increased voided volume, and the ratio of notch-like intravesical pressure reduction durations to the voiding contraction time was significantly prolonged. In 30-day SCI mice with 27-day inhibition of BDNF, voiding efficiency was improved with decreased PVR, the ratio of notch-like reduction duration to the voiding contraction time was significantly prolonged, and the number of NVC was significantly decreased. $^{\#}P < .05$ vs 10-day SCI mice, $^{\ddagger}P < .05$ vs 30-day SCI mice. B, Comparison of urodynamic parameters in 10-day or 30-day SCI mice with or without anti-BDNF treatment. BDNF, brain-derived neurotrophic factor; NVC, non-voiding contractions; PVR, post-void residual; SCI, spinal cord injury

would be necessary to significantly reduce C-fiber-dependent DO evident as decreased NVC during the storage phase in SCI mice. Previous studies have shown that NGF is another important neurotrophin that contributes to DO after SCI[3,8] although anti-NGF Ab treatment, which was started at 2 to 3 weeks after SCI, induced a significant, but only partial reduction of NVC during bladder filling in SCI mice.[8] In the current study, we found the similar partial reduction of NVC after BDNF inhibition although the early-started, long-term anti-BDNF treatment was required in contrast to the short-term, late-phase anti-NGF treatment in a previous study.[6] Thus, it is possible that NGF and BDNF independently or interactively function in different phases to induce DO during bladder filling after

SCI, however, further studies are needed to clarify this issue.

In mice, voiding efficiency was quite low throughout the post-SCI periods as similarly shown in previous reports.[6-8] According to the previous research findings, SCI rats have EUS bursting during bladder contractions, which produces the pumping activity of the urethra, leading to efficient voiding. However, most normal mice exhibit reduced EUS activity without bursting during voiding, which is similar in humans, and in SCI mice, the urethral pumping activity does not emerge, resulting in much lower voiding efficiency compared to SCI rats.[7] Low voiding efficiency was induced as a result of quite small voided volume at 10 days and increased PVR and bladder capacity at 20 and 30 days after SCI, as revealed in this study. In SCI mice, bladder mucosal BDNF was higher at each time point compared to spinal intact mice, and most highly expressed in the early phase (day 5) after SCI. This is consistent with previous findings that mRNA levels of BDNF in the bladder elevated 78-fold for only 4 days after SCI in rats.[20,21] It is possible that a higher level of bladder BDNF in the early phase might be necessary for recovery of the micturition reflex from the shock phase; however, both early-started, short- and long-term inhibitions of BDNF by anti-BDNF Ab did not adversely influence the recovery of the micturition reflex or voiding function, but improved voiding efficiency.

In the previous study, the late-phase, short-term (7 days) inhibition of BDNF increased voiding efficiency and improved synergistic activity of EUS using EUS-EMG evaluation without affecting NVC during the storage phase.[6] Also, the CMG-EMG evaluation in SCI mice revealed that notch-like intravesical pressure reductions on CMG traces coincided with the periods of reduced EMG activity during voiding contraction and, during these periods, actual voids occurred.[6,7] A prolongation of periods of reduced EMG activity during voiding could imply an increased synergistic relaxation of EUS. Taken together, it is assumed that the evaluation of notch-like reduction periods on CMG can be used as a surrogate marker to evaluate synergistic activity of EUS during voiding. Based on the current study results, early-started, short- or long-term inhibition of BDNF, increased voiding efficiency, and improved synergistic activity of EUS during bladder contractions, evident as the prolonged ratio of notch-like reduction durations to voiding contraction time on CMG traces. These findings suggest that BDNF is involved in SCI-induced voiding dysfunction due to dyssynergic activity of EUS, that is, DSD, throughout the post-SCI period up to 30 days.

The major aims of the current study were to observe the time-course urodynamic changes and to examine the effect of early-started inhibition of BDNF on the bladder storage and voiding dysfunction in SCI mice. However, there are some limitations to this study. First, even though our recent study suggested that overexpressed BDNF enhanced the bladder-to-EUS reflex after SCI via activation of mechanosensitive bladder afferent pathways to induce DSD,[6] further studies are needed to clarify how BDNF is mechanistically involved in SCI-induced EUS dyssynergic activity during voiding. Secondly, in this study, anti-BDNF Ab was administered systemically in SCI mice. Therefore, further research, for example, using intrathecal or intravesical administration, is needed to determine the site of action of BDNF. Finally, the molecular mechanisms underlying SCI-induced urethral and afferent dysfunctions are still not known; thus, they need to be investigated in future studies.

5 | CONCLUSIONS

In SCI mice, overexpressed BDNF in the bladder is involved in inefficient voiding and also in the emergence of NVC during the storage phase. The early-started, short- and long-term inhibition of BDNF both improved voiding dysfunction associated with DSD; however, the long-term BDNF inhibition was required to reduce the later-phase development of DO after SCI.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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