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Research Paper

Complete spinal cord injury (SCI) transforms how brain derived neurotrophic factor (BDNF) affects nociceptive sensitization

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ABSTRACT

Noxious stimulation can induce a lasting increase in neural excitability within the spinal cord (central sensitization) that can promote pain and disrupt adaptive function (maladaptive plasticity). Brain-derived neurotrophic factor (BDNF) is known to regulate the development of plasticity and has been shown to impact the development of spinally-mediated central sensitization. The latter effect has been linked to an alteration in GABA-dependent inhibition. Prior studies have shown that, in spinally transected rats, exposure to regular (fixed spaced) stimulation can counter the development of maladaptive plasticity and have linked this effect to an up-regulation of BDNF. Here it is shown that application of the irritant capsaicin to one hind paw induces enhanced mechanical reactivity (EMR) after spinal cord injury (SCI) and that the induction of this effect is blocked by pretreatment with fixed spaced shock. This protective effect was eliminated if rats were pretreated with the BDNF sequestering antibody TrkB-IgG. Intrathecal (i.t.) application of BDNF prevented, but did not reverse, capsaicin-induced EMR. BDNF also attenuated cellular indices (ERK and pERK expression) of central sensitization after SCI. In uninjured rats, i.t. BDNF enhanced, rather than attenuated, capsaicin-induced EMR and ERK/pERK expression. These opposing effects were related to a transformation in GABA function. In uninjured rats, BDNF reduced membrane-bound KCC2 and the inhibitory effect of the $GABA_A$ agonist muscimol. After SCI, BDNF increased KCC2 expression, which would help restore GABAergic inhibition. The results suggest that SCI transforms how BDNF affects GABA function and imply that the clinical usefulness of BDNF will depend upon the extent of fiber sparing.

1. Introduction

Over the last two decades, there has been a plethora of studies examining how brain-derived neurotrophic factor (BDNF) affects neural function (for reviews, see Boyce and Mendell, 2014; Cunha et al., 2010; Hollis and Tuszynski, 2011; Merighi et al., 2008; Smith, 2014; Waterhouse and Xu, 2009). Interest in this protein has been fueled by evidence that it promotes neuronal growth, synaptogenesis, cell survival and neurogenesis (Smith, 2014; Weishaupt et al., 2012). At a functional level, BDNF has been implicated in learning and memory, with research demonstrating that it is released in an activity dependent manner, promotes the development of long-term potentiation (LTP), and fosters memory formation (Cunha et al., 2010; Waterhouse and Xu, 2009). The action of BDNF has been linked to the TrkB receptor and its downstream effectors, including phospholipase C (PLC), extracellular signal-regulated kinase (ERK), and Akt, which have been tied to synaptic plasticity, axonal growth, and cell survival, respectively (Minichiello, 2009). Interestingly, how BDNF affects neural function appears to be regulated by the cellular environment, leading some to suggest that it acts to maintain a form of homeostasis that promotes adaptive plasticity (Swanwick et al., 2006; Wenner, 2014).

These findings have led researchers to posit that BDNF could foster cell survival and adaptive plasticity after spinal cord injury (SCI; Boyce and Mendell, 2014; Hollis and Tuszynski, 2011; Weishaupt et al., 2012). BDNF has been shown to enhance axonal growth, enable locomotor function in spinally transected animals, and promote respiratory function after cervical injury (Baker-Herman et al., 2004; Boyce et al., 2012; Boyce et al., 2007; Cote et al., 2011; Tashiro et al., 2015; Vaynman and Gomez-Pinilla, 2005). In addition, both locomotor training and spinally-mediated learning have been shown to up-regulate BDNF expression after SCI (Gomez-Pinilla et al., 2007; Gomez-Pinilla et al., 2001; Huie et al., 2012). Exogenously applied BDNF, microinjected by

Abbreviations: aCSF, artificial cerebrospinal fluid; BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; Cap, capsaicin; CNS, central nervous system; EMR, enhanced mechanical reactivity; ERK, extracellular signal-regulated kinases; GABA, Gamma-aminobutyric acid; i.t., intrathecal; KCC2, K^+-Cl^- cotransporter 2; LTP, long-term potentiation; MAPK, mitogen-activated protein kinases; Mus, muscimol; N-CAD, N-cadherin; NKCC1, Na^+-K^+-Cl^- cotransporter 1; NMDAR, *N*-methyl-D-aspartate receptor; pERK, phosphorylated ERK; PLC-γ, phospholipase C-γ; SCI, spinal cord injury; Shk, shock; 5HT, serotonin; T2, second thoracic; TrkB, tropomyosin receptor kinase B; Unshk, unshock; Veh, vehicle.

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means of an intrathecal (i.t.) catheter, has been shown to foster spinal learning and block the learning impairment induced by variable intermittent stimulation (shock) applied at an intensity that engages pain (C) fibers (Gomez-Pinilla et al., 2007; Huie et al., 2012). Interestingly, spinal learning is also disrupted by treatments that induce nociceptive sensitization within the spinal cord (e.g., the peripheral application of the irritant capsaicin) that manifests behaviorally as enhanced mechanical reactivity (EMR; Ferguson et al., 2006; Ferguson et al., 2012a; Ferguson et al., 2012b; Hook and Grau, 2007). Prior work has shown that the induction of nociceptive sensitization, and the associated spinal learning impairment, are inhibited by training with either response-contingent or temporally predictable stimulation (Baumbauer and Grau, 2011; Baumbauer et al., 2012; Hook and Grau, 2007). We have linked the restoration of spinal learning to the release of BDNF, demonstrating that the protective effect of training is blocked by pretreatment with the BDNF sequestering antibody TrkB-IgG (Baumbauer et al., 2009; Huie et al., 2012). The present paper extends these findings by testing whether TrkB-IgG also blocks the protective effect of training on nociceptive sensitization. Conversely, we explore whether pretreatment with BDNF inhibits the development of capsaicin-induced EMR in spinally transected rats.

The idea that BDNF may counter the development of spinally-mediated nociceptive sensitization after SCI runs counter to a large literature implicating this ligand in the development of central sensitization (Merighi et al., 2008; Pezet et al., 2002; Smith, 2014). Prior work has shown that treatments that increase spinal BDNF in uninjured animals induce EMR (Beggs and Salter, 2013; Coull et al., 2005; Garraway et al., 2003; Kerr et al., 1999; Latremoliere and Woolf, 2009; Lu et al., 2009; Merighi et al., 2008; Miletic and Miletic, 2008; Thompson et al., 1999). Conversely, the EMR induced by the application of peripheral irritants (e.g., formalin) is inhibited by TrkB-IgG (Kerr et al., 1999). Further, electrophysiological studies indicate that the application of BDNF can sensitize nociceptive activity in lamina II of the dorsal horn (Garraway et al., 2003). These pronociceptive effects have been tied to a BDNF-induced reduction in GABAergic inhibition linked to the down-regulation of membrane-bound K^+-Cl^- cotransporter 2 (KCC2; Beggs and Salter, 2010; Coull et al., 2005; Smith, 2014). How BDNF affects KCC2 and neuronal excitability within the dorsal horn appears to be regulated by descending fibers (Boulenguez et al., 2010; Garraway et al., 2005; Garraway and Mendell, 2007; Shulga et al., 2008). By interrupting these fibers, SCI could potentially transform how BDNF affects nociceptive sensitization. We show that SCI not only attenuates the pronociceptive effect of BDNF, SCI reverses its action, unveiling an antinociceptive effect that inhibits the development of capsaicin-induced EMR. We relate this transformation to a BDNF-induced increase in KCC2 and GABAergic inhibition.

2. Experimental procedures

2.1. Subjects

Subjects were male Sprague-Dawley rats obtained from Envigo (Houston, TX) that were 80–100 days old, and between 325 and 400 g. All subjects were pair housed and maintained on a 12-hour light/dark cycle, with all behavioral testing performed during the light cycle. Food and water was available ad libitum. All experiments were carried out in accordance with NIH standards for the care and use of laboratory animals (NIH publications No. 80-23), and were approved by the University Laboratory Animal Care Committee at Texas A&M University. Every effort was made to minimize suffering and limit the number of animals used.

2.2. Surgery

Prior to surgery, the fur over the thoracic portion of the vertebral column was shaved and disinfected with betadine solution. Subjects were anesthetized with isoflurane gas. Anesthesia was induced at 5% isoflurane and maintained at 2–3% isoflurane. Each subject's head was rendered immobile in a stereotaxic apparatus, and a small (5 × 4 × 2.5 cm) gauze pillow was placed under the subject's chest to provide support for respiration. An anterior to posterior incision over the second thoracic vertebrae (T2) was made and the tissue just rostral to T2 was cleared using rongeurs, and the cord was exposed. A cautery device was then used to transect the cord and a 25-cm polyethylene cannula (PE-10, VWR International, Bristol, CT, USA) was subsequently threaded 9 cm down the vertebral column, into the subarachnoid space between the dura and the white matter so that it lay on the dorsal surface of the spinal cord. After surgery, the incision was closed using Michel clips (Fine Science Tools, Foster, CA, USA), and the exposed end of cannula tubing was fixed to the skin with cyanoacrylate.

Following surgery, rats were placed in a temperature-controlled environment (25.5 °C) and monitored until awake. All rats were checked every six to eight hours during the 18–24 h post-surgical period. During this time, hydration was maintained with supplemental injections of saline, and the rats' bladders and colons were expressed as needed. As in prior studies (Garraway et al., 2014; Huie et al., 2012; Lee et al., 2016; Lee et al., 2015), testing began 24 h after surgery.

Spinal transections were confirmed by inspecting the cord at the time of surgery and observing the behavior of the subjects after they recovered to ensure that they exhibited paralysis below the level of the forepaws and did not exhibit any brain-dependent responses to stimulation caudal to injury.

2.3. Drug administration

Drugs were administered intrathecally (i.t.) using a 10 μL Hamilton syringe attached to the exposed end of each subject's i.t. cannula. BDNF (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 10 μL of artificial cerebrospinal fluid (aCSF) + 0.1% bovine serum albumin (BSA) vehicle. The BDNF sequestering agent TrkB-IgG (R&D Systems, Minneapolis, MN) was also injected intrathecally, as described above. Muscimol (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.9% saline. Each compound was administered at a constant rate over a period of several minutes. Following the injection, the cannula was flushed with 20 μL of 0.9% saline.

2.4. Fixed spaced leg shock

All fixed spaced (ISI: 2 s) shock was administered to one hind leg. An electrode was inserted through the skin over the distal portion of the tibialis anterior muscle (1.5 cm from the plantar surface of the foot). Next, the proximal portion of the tibialis anterior (approximately 1.7 cm proximal to the wire electrode) was probed with a 2.5-cm stainless steel pin attached to a shock lead to locate a position that elicited a robust flexion response. The pin was then inserted 0.4 cm into the muscle. Shock intensity was set to 0.4 mA shock,

2.5. Mechanical testing

Mechanical reactivity was assessed using von Frey filaments (Stoelting, Wood Dale, IL) that were applied while rats were loosely restrained in Plexiglas tubes. Sensitivity was determined by applying calibrated filaments to the mid-plantar surface of each hindpaw in an ascending order until a flexion response was elicited. Stimuli were presented twice to each paw in an ABBA counterbalanced fashion (A = left, B = right), with testing on the same leg separated by a 2 min interval. Filament thickness/force was related to behavior using the transformation provided by the manufacturer: Intensity = log10 (10,000 g). This transformation yields a scale that is approximately linear and amenable to parametric analyses. Data were converted to change from baseline scores for purposes of analysis. The experimenter performing the behavioral tests was unaware of the subject's treatment condition.

2.6. Capsaicin injections

Capsaicin (Sigma-Aldrich, St. Louis, MO) was dissolved in 50 μL of vehicle (Tween 20 [7%] and saline [93%]) and a 1% solution was injected subcutaneously into the dorsal surface of the paw. Using the dorsal surface of the paw helped ensure that the capsaicin-induced edema did not affect the assessment of mechanical reactivity.

2.7. Western blotting

Tissue was collected for Western blotting immediately following mechanical testing. Subjects were deeply anesthetized with a lethal dosage of pentobarbital (140 mg/kg) and a 1-cm portion of the spinal cord containing the lumbosacral enlargement was removed and rapidly frozen. The spinal cord was further subdivided into dorsal and ventral portions and total protein was extracted using the QIAzol™ lysis reagent protocol Hummon et al. (2007) used in prior studies (e.g., Garraway et al., 2014; Qiagen, Valencia, CA). After determining the protein concentration by Bradford Assay (BioRad, Hercules, CA), protein samples were diluted in Laemmli sample buffer and were stored at −80 °C at known concentrations (usually 2–5 μg/μL). Western blotting was used for the protein quantification of ERK44/42 and pERK44/42 (~44/42 kDa). Equal amounts (30 μg) of total protein were subjected to SDS-PAGE with 12% Tris-HEPES precast gels (Pierce, Rockford, IL). After transferring onto PVDF membranes (Millipore, Bedford, MA) by Bio-Rad Semi-dry transfer apparatus, the blots for ERK44/42 and other non-phosphorylated proteins (see below) were blocked for 1 h in 5% blotting grade milk (BioRad, Hercules, CA) in Tris-Buffered Saline Tween-20 (TBST), while blots for pERK44/42 were blocked in 5% BSA in TBST. After blocking, the PVDF membranes were incubated overnight at 4 °C in one of the following primary antibodies generated in rabbit: ERK44/42 (1:2000; #06-182 - Millipore, Temecula, CA), pERK44/42 (1:500; #07-467 - Millipore, Temecula, CA). β-Actin (1:2500; #Ab8227 - Abcam, Cambridge, MA) served as the control. All primary antibodies were diluted in blocking solution. The following day, PVDF membranes were washed in TBST (3 × 5 min) at room temperature and incubated in HRP-conjugated goat anti-rabbit or anti-mouse secondary antibodies (1:5000; #31460 or 31430, respectively; Pierce, Rockford, IL) for 1 h at room temperature. After another 3 × 5 min series of washes, the blots were incubated with ECL (Pierce, Rockford, IL) and were imaged with Fluorchem HD2 (ProteinSimple, Santa Clara, CA). The protein expression for each gene of interest was normalized to β-actin expression and presented as a fold change relative to the sham controls. Other targets of interest: KCC2 (1:500; #07-432 - Millipore, Temecula, CA) and N-cadherin (1:1000; cell signaling - Danvers, MA) were assessed in the same fashion.

2.8. Fractionation

To assess KCC2 (1:500; #07-432 - Millipore, Temecula, CA) expression, spinal cord specimens were homogenized with dounce homogenizer (Kontes), followed by 5 passes through a 22 gauge needle in ice-cold buffer, pH 7.5, containing 10 mm Tris, 300 mm sucrose, and a complete mini protease inhibitor mixture (Roche). Crude homogenates were centrifuged at 5000 RCF for 5 min at 4 °C. Supernatant was further fractionated at 13,000 RCF for 30 min. After centrifugation, supernatant was collected as cytoplasmic fraction. A membrane rich fraction was then obtained by resuspending the pellet in PBS (50 μL) containing protease inhibitor. All samples were sonicated and stored at −80 °C for later processing (Western blotting). N-cadherin (1:1000; Cell Signaling - Danvers, MA) was used to confirm plasma membrane enrichment.

2.9. Statistics

All data were analyzed using repeated measures analysis of variance (ANOVA) or analysis of covariance (ANCOVA). Differences between group means were assessed using Duncan's New Multiple Range post hoc tests when necessary.

We controlled for individual variability in baseline reactivity in two ways: (1) By analyzing the test data using an ANCOVA, entering the baseline score as a covariate; and (2) By computing a change from baseline score and analyzing the data using an ANOVA. An advantage of the latter is that our index of variability [the standard error of the mean (SE)] is computed after we adjust for individual differences. Because this simplifies the assessment of group differences, and because an ANCOVA performed on the raw scores and an ANOVA conducted on the change from baseline values yielded the same pattern of statistical significance across experiments, we present just the change from baseline scores. In all cases, $p < 0.05$ was used to determine statistical significance.

3. Results

3.1. Experiment 1: TrkB-IgG blocks the protective effect of fixed spaced stimulation

Prior work has shown that the impact of nociceptive stimulation on spinal function is regulated by learning (Baumbauer and Grau, 2011; Grau et al., 2006; Grau et al., 2012; Grau et al., 2014; Hook et al., 2008). In spinally transected rats, exposure to uncontrollable/unpredictable intermittent shock, at an intensity that engages pain (C) fibers, impairs spinal learning and enhances reactivity to mechanical stimulation (Ferguson et al., 2003; Grau et al., 2014). Treatments that induce spinally-mediated nociceptive sensitization (e.g., peripheral application of capsaicin) also impair spinal learning. Prior work has shown that exposure to 720–900 fixed spaced shocks induces a lasting protective effect that blocks the induction of the spinal learning impairment induced by variable unpredictable stimulation or capsaicin (Baumbauer and Grau, 2011; Baumbauer et al., 2009; Lee et al., 2016; Lee et al., 2015). This protective effect is blocked by pretreatment with the BDNF sequestering antibody TrkB-IgG. Fixed spaced stimulation also attenuates the development of capsaicin-induced EMR (Baumbauer and Grau, 2011). The present experiment tests whether this effect is also blocked by TrkB-IgG.

Twenty-four subjects (n = 6 per condition) received baseline assessments of tactile reactivity followed by an i.t. infusion of 9.0 pmol (10 μL vol) of the BDNF sequestering molecule TrkB-IgG or saline (see Fig. 1). Catheters were then flushed with 20 μL of 0.9% saline. This dose has previously been shown to block the effect of fixed spaced stimulation (Baumbauer et al., 2009). Fifteen minutes later, rats were administered 0 or 900 fixed spaced shocks, followed by another assessment of tactile reactivity (Post shock). All rats were then given subcutaneous injections of 1% capsaicin (50 μL vol) into one hindpaw. Leg shock and capsaicin were administered on the same limb and the leg treated was counterbalanced across subjects. Finally, tactile reactivity was recorded, 30, 60, and 180 min following treatment with capsaicin (Post Capsaicin).

Mean baseline tactile reactivity scores ranged from 6.05 ± 0.02 to 6.08 ± 0.01 and did not differ, all Fs < 1.00, $p > 0.05$. After shock treatment, we found that unshocked animals, irrespective of drug condition, exhibited no change in tactile reactivity, while rats given fixed stimulation and saline were less responsive (Fig. 1A). Treatment with TrkB-IgG attenuated the shock-induced change in behavior. An ANOVA confirmed that the main effects of drug and shock treatment were significant, both F's > 11.22, $p < 0.005$. In addition, there was a significant Drug × Shock interaction, $F_{(1, 20)} = 8.48$, $p < 0.01$. Post hoc comparisons of the group means confirmed that rats given saline and fixed spaced stimulation had significantly higher response thresholds when compared to animals in all other conditions ($p < 0.05$). No other comparisons reached statistical significance ($p > 0.05$).

As expected, treatment with capsaicin enhanced reactivity to mechanical stimulation in the unshocked controls (Fig. 1B & C). Exposure

Complete transection (T2)	24 hr	von Frey baseline	Vehicle	15 min	Unshock	von Frey	Capsaicin	von Frey 30, 60, 180 min
			TrkB-IgG		FT Shock			

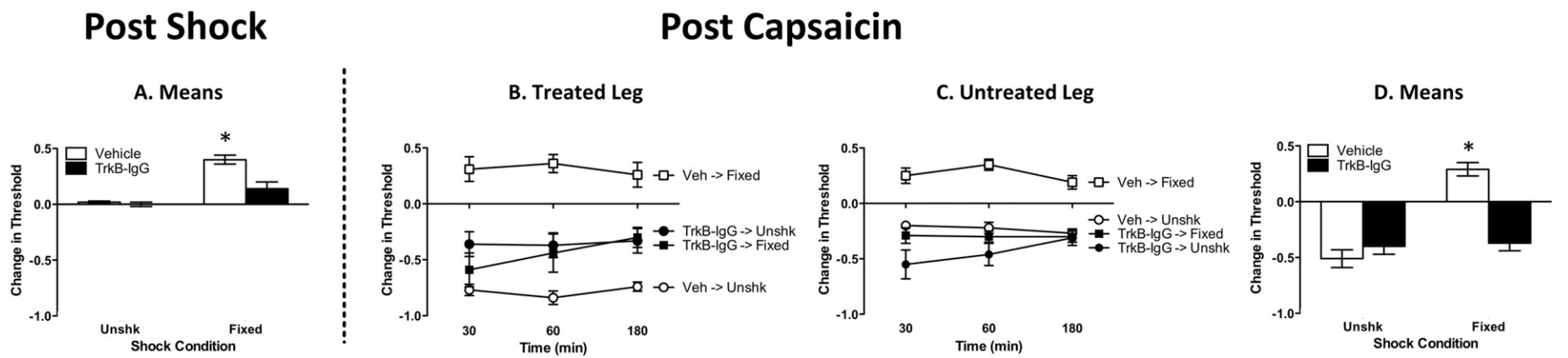


Fig. 1. TrkB-IgG blocks the protective effect of fixed spaced stimulation on capsaicin-induced EMR in spinally transected subjects. The experimental design is illustrated at the top of the figure. A day after spinal transection, rats received TrkB-IgG (i.t.; filled symbols) or vehicle (unfilled). Fifteen min later, subjects received fixed-space shock (fixed; squares) or no shock (Unshk; circles) to one hind paw. All rats were then given a subcutaneous injection of capsaicin into the same hindpaw. Mechanical reactivity was assessed prior to TrkB-IgG (baseline) and capsaicin treatment (Post Shock) and 30, 60, and 180 min after capsaicin (Post Capsaicin). The y-axis depicts linearized mechanical reactivity scores [log 10 (10,000 g)] as a change from baseline. (A) Effect of drug and shock treatment on mechanical reactivity (collapsed across legs). (B) The change in mechanical reactivity post capsaicin for the treated leg. (C) The change from baseline scores for the untreated leg. (D) Mean reactivity (collapsed across time and leg). The error bars indicate the standard error of the mean (SEM). * Indicates statistical significance ($p < 0.05$).

to fixed spaced stimulation blocked this effect in vehicle treated, but not TrkB-IgG treated, rats. An ANOVA showed that the main effects of drug, shock, and leg, were significant, all F's > 7.68, $p < 0.05$. In addition, the Drug × Shock and Drug × Shock × Leg interactions were all significant, all Fs > 17.96 < 0.001. Post hoc comparisons of the group means (collapsed across test leg; Fig. 1D) showed that the Saline treated rats given fixed spaced shock differed from the other three groups ($p < 0.05$). No other group differences were significant ($p > 0.05$).

3.2. Experiment 2: BDNF attenuates capsaicin-induced EMR in spinally transected subjects

Experiment 1 replicates past work demonstrating that fixed spaced stimulation attenuates capsaicin-induced EMR (Baumbauer and Grau, 2011) and showed that this effect is blocked by the BDNF sequestering antibody TrkB-IgG, suggesting that BDNF plays an essential (necessary) role. Our next experiment addresses the issue of sufficiency by testing whether i.t. treatment with BDNF can substitute for fixed spaced stimulation. We hypothesized that BDNF would block the induction of capsaicin-induced EMR in spinally transected rats.

The experimental design is illustrated at the top of Fig. 2. Thirty-two spinally transected subjects (n = 8) received baseline assessments of mechanical reactivity 24 h after a complete transection at T2. Immediately after baseline assessment, half of the subjects were given an i.t. infusion of BDNF (0.4 μg; 10 μL volume) and half were given an equivalent amount of vehicle. Catheters were then flushed with 20 μL of 0.9% saline, followed 30 min later by a subcutaneous injection of 1% capsaicin to the dorsal surface of the paw. The leg treated with capsaicin was counterbalanced across subjects. Mechanical reactivity was assessed two times each on the treated and untreated leg (in an ABBA order, counter-balanced across subjects) over a period of 8 min after capsaicin treatment (0 h test). Testing was repeated at 1, 2, and 3 h.

Mean baseline mechanical reactivity scores ranged from 5.9 ± 0.1 to 6.1 ± 0.2 (mean ± SE) and did not differ, all Fs < 3.39, $p > 0.05$. Rats that received capsaicin alone (Veh- > Cap) were more responsive to mechanical stimulation across the 3 h of testing (Fig. 2A & B). This EMR was blocked by pretreatment with BDNF (BDNF- > Cap). An ANOVA confirmed that the main effect of drug and capsaicin treatment were significant, both F's > 19.93, $p < 0.001$. In addition, there was a significant Drug × Capsaicin interaction, $F_{(1, 28)} = 11.14$, $p < 0.01$. A stronger effect was observed on the treated leg (Fig. 2A versus 2B), yielding both a main effect of leg, $F_{(1, 28)} = 21.97$, $p < 0.001$, as well as significant Leg × Drug and Leg × Capsaicin interactions, F's > 5.81, $p < 0.05$. Post hoc comparisons of the group means (Fig. 2C) confirmed that rats given vehicle and then capsaicin had significantly lower response thresholds relative to the other conditions ($p < 0.05$).

Here, and in the remaining experiments, the EMR observed on the untreated leg was somewhat weaker. However, the pattern of results was unchanged. Because this remained true in all of the subsequent experiments, we focus on the averaged data (collapsed across test legs) in subsequent experiments.

3.3. Experiment 3: BDNF does not reverse capsaicin-induced EMR

Experiment 2 showed that pretreatment with BDNF can block the development of spinally-mediated nociceptive sensitization. Experiment 3 examined whether BDNF can reverse the EMR after it is induced.

The design is depicted at the top of Fig. 3. After baseline mechanical reactivity was assessed, one group (BDNF- > Cap; n = 8) received BDNF (0.4 μg) followed by a 20 μL of 0.9% saline. Thirty minutes later, rats were treated with capsaicin and mechanical reactivity was re-tested 0, 1, 2, and 3 h later as described above. A second group (Cap- > BDNF; n = 8) was treated with BDNF immediately after the 1 h test. The final group (Cap Alone; n = 8) received vehicle rather than BDNF.

Mean baseline mechanical reactivity scores ranged from 6.01 ± 0.1 to 6.07 ± 0.1 and did not differ, $p > 0.05$. As expected, capsaicin treatment (Cap Alone) induced a lasting EMR (Fig. 3A). This EMR was attenuated by BDNF given prior to capsaicin (BDNF- > Cap). Administration of BDNF 1 h after capsaicin treatment (Cap- > BDNF) had no effect. An ANOVA confirmed that there was a main effect of treatment condition, $F_{(2,21)} = 11.71$, $p < 0.001$, as well as a main effect of leg, $F_{(1, 21)} = 83.82$, $p < 0.001$. Post hoc comparisons of the group means (Fig. 3B) showed that the group given BDNF prior to capsaicin treatment (BDNF- > Cap) differed from the other groups ($p < 0.05$). No other group differences were significant ($p > 0.05$).

3.4. Experiment 4: Spinal cord injury transforms how BDNF affects nociceptive sensitization

Here we have shown that exogenous application of BDNF can block (but not reverse) the induction of capsaicin-induced EMR in spinally transected rats. However, other studies suggest that BDNF fosters the

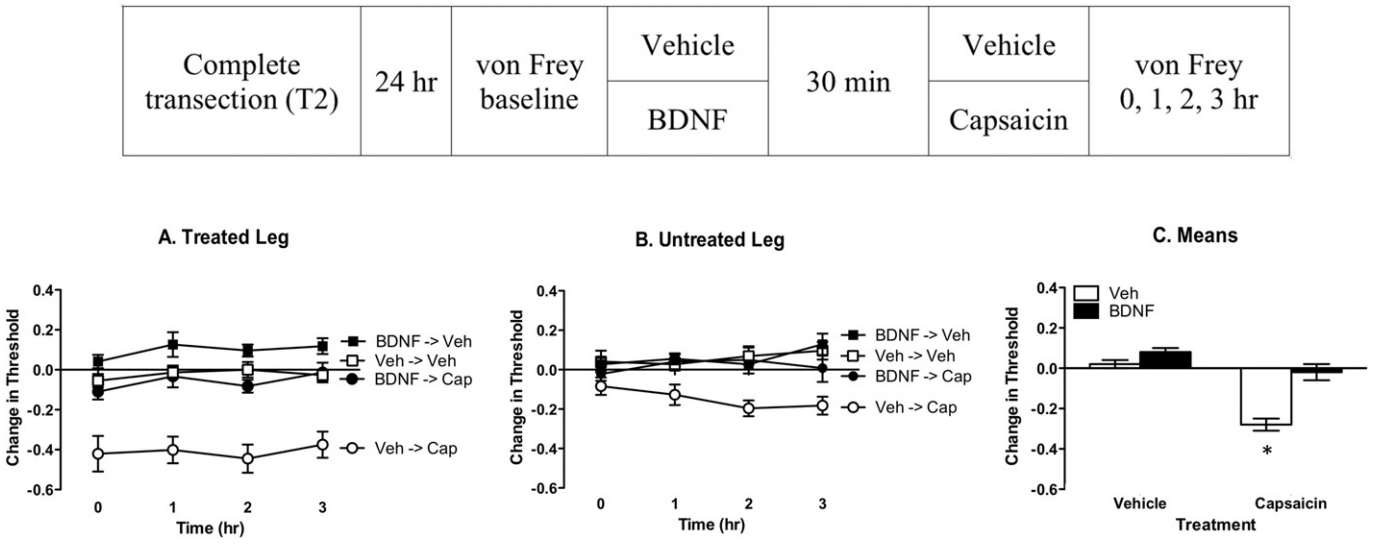


Fig. 2. BDNF attenuates capsaicin-induced EMR in spinally transected subjects. The experimental design is illustrated at the top of the figure. A day after spinal transection, rats received BDNF (i.t.; filled symbols) or vehicle (unfilled). Thirty minutes later, capsaicin (circles) or its vehicle (squares) was applied to the dorsal surface of one hind paw. The y-axis depicts linearized mechanical scores [log 10 (10,000 g)] as a change from baseline score. (A) Mechanical reactivity after capsaicin for the treated leg. (B) The change from baseline scores for the untreated leg. (C) Mean reactivity (collapsed across time and leg). The error bars indicate the standard error of the mean (SEM). * Indicates statistical significance ($p < 0.05$).

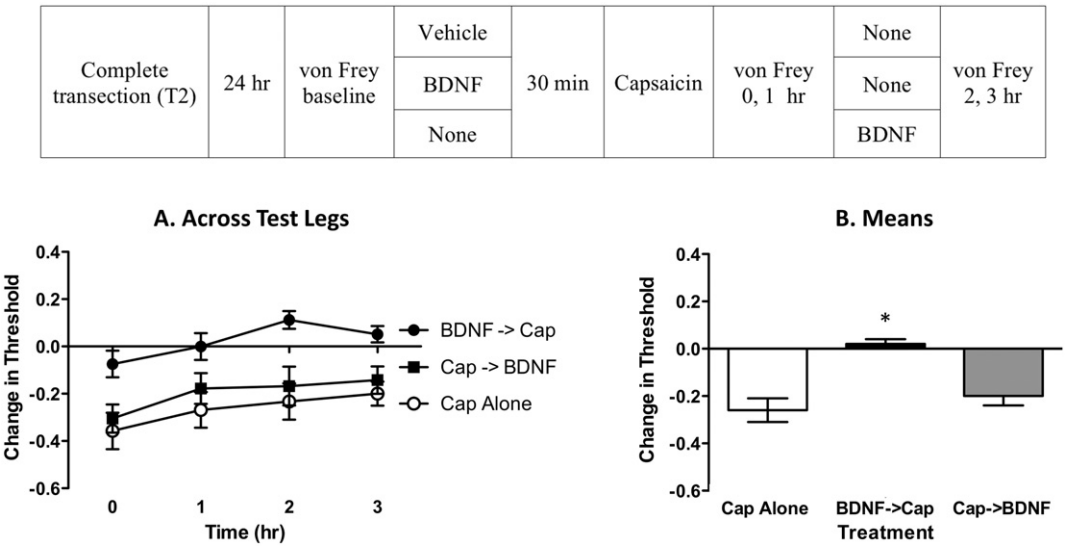
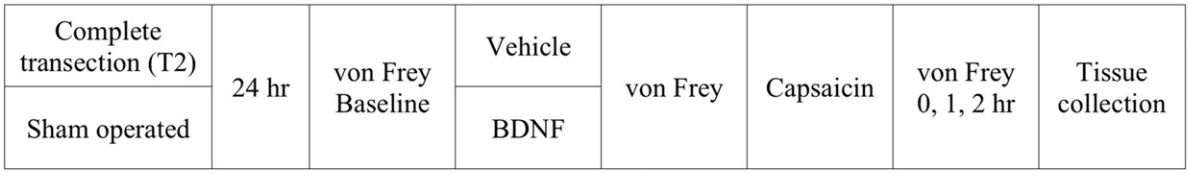


Fig. 3. BDNF does not reverse capsaicin-induced EMR. The experimental design is illustrated at the top of the figure. A day after spinal transection, one group of rats received BDNF (i.t.; filled circles) and 30 min later capsaicin was applied to the dorsal surface of one hind paw. A second group received BDNF an hour after capsaicin treatment (filled squares). A third group received vehicle, either prior to or 1 h after capsaicin treatment (counterbalanced; open circles). The y-axis depicts linearized mechanical reactivity scores [log 10 (10,000 g)] as a change from baseline. (A) Mechanical reactivity over time after capsaicin treatment (collapsed across legs). (B) Mean reactivity (collapsed across time and leg). The error bars indicate the standard error of the mean (SEM). * Indicates statistical significance ($p < 0.05$).

development of nociceptive sensitization (Merighi et al., 2008; Miki et al., 2000). As noted elsewhere (Grau et al., 2012), the divergent effects of BDNF may be linked to differences in drug concentration or the behavioral assay employed. Here, we explore a third possibility, that the impact of BDNF on nociceptive processing within the spinal cord is modulated by spinal cord injury. Prior work has shown that descending fibers regulate the physiological state of the spinal cord and impact ion-channel-mediated plasticity (Crown and Grau, 2005; Ferrini and De Koninck, 2013; Gjerstad et al., 2001; Medina et al., 2014). Spinal cord injury can disrupt this modulatory effect, reinstating a neurochemical environment caudal to injury that resembles an early development (Boulenguez et al., 2010; Cramer et al., 2008; Lu et al., 2008; Wenner, 2014). Given these observations, we hypothesized that spinal cord injury may transform how BDNF affects nociceptive sensitization. We evaluate this possibility by testing the impact of i.t. BDNF on capsaicin-induced EMR in spinally transected and sham-operated rats. We reinforce our behavioral observations with cellular data by assaying ERK44/42 (extracellular signal-regulated kinases 44 and 42) and pERK (phosphorylated extracellular signal-regulated kinases) protein expression within dorsal horn. Prior work has shown that these ligands are



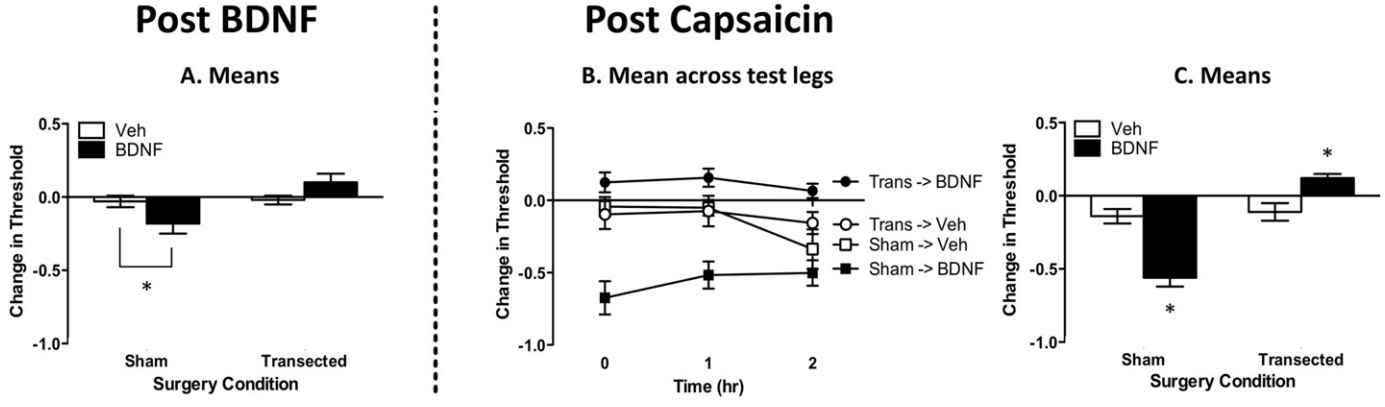


Fig. 4. Spinal cord injury transforms how BDNF affects nociceptive sensitization. The experimental design is illustrated at the top. Subjects received either a spinal transection (Trans; circles) or a sham surgery (Sham; squares). The next day, BDNF (filled symbols) or vehicle (unfilled symbols) was administered (i.t.) followed by capsaicin. The y-axis depicts linearized mechanical reactivity scores [log 10 (10,000 g)] as a change from baseline. (A) Effect of surgery and BDNF treatment on mechanical reactivity (collapsed across leg). (B) The change from baseline scores post capsaicin (collapsed across leg). (C) Mean reactivity (collapsed across time and leg). The error bars indicate the standard error of the mean (SEM). * Indicates statistical significance ($p < 0.05$).

engaged by peripheral inflammation and that their expression is linked to central sensitization (Adwanikar et al, 2004; Gao and Ji, 2009).

The experimental design is illustrated at the top of Fig. 4. Twenty-four subjects (n = 6 per condition) received either a full transection at the second thoracic vertebrae or a sham surgery. Twenty-four hours later, half of the subjects were given an intrathecal injection of BDNF (0.4 μg) and half were administered vehicle. Catheters were then flushed with 20 μL of 0.9% saline. Fifteen minutes after BDNF or vehicle injection, all subjects received a subcutaneous injection of capsaicin (1%). Mechanical reactivity was assessed immediately before BDNF injection (baseline), before capsaicin injection, and 0, 1, and 2 h post capsaicin injection. At the end of behavioral testing, the subjects were euthanized and the L3–L5 tissue was collected. Western blotting was then used to assay ERK 44/42 (both ERK and pERK).

Prior to BDNF and capsaicin treatment, sham-operated subjects were more responsive to mechanical stimulation (sham = 5.83 ± 0.06; transected = 6.02 ± 0.04 [mean ± SE]), $F_{(1, 22)} = 6.59$, $p < 0.05$. BDNF treatment (Post BDNF) enhanced mechanical reactivity in sham-operated (Fig. 4A), but not transected, rats. An ANOVA confirmed that the effect of BDNF treatment depended upon surgery condition, $F_{(1, 20)} = 5.03$, $p < 0.05$.

Capsaicin (Post Capsaicin) induced a moderate EMR in vehicle-treated rats regardless of surgical condition (Sham- > Veh, Trans- > Veh) that grew stronger over time (Fig. 4B & C). An ANOVA confirmed that these groups were more responsive to mechanical stimulation after capsaicin treatment (relative to baseline), $F_{(1,10)} = 9.94$, $p < 0.05$. In sham-operated rats, pretreatment with BDNF (Sham- > BDNF) enhanced capsaicin-induced EMR. In injured rats, BDNF had the opposite effect (Trans- > BDNF). An ANOVA confirmed that there was a main effect of surgery and a significant Surgery × Drug treatment interaction, both F's > 33.86, $p < 0.0001$. Post hoc comparisons showed that sham-operated rats given BDNF differed from the other groups ($p < 0.05$). Likewise, transected rats given BDNF differed from the other three conditions ($p < 0.05$). No other comparisons were significant ($p > 0.05$).

BDNF also showed opposite effects on ERK44/42 and pERK44/42 protein expression in sham-operated and transected subjects (Fig. 5). In sham-operated rats, BDNF treatment increased protein expression of ERK44/42 and pERK44/42, which suggests that BDNF enhances capsaicin-induced central sensitization. As reported elsewhere (Huang et al., 2014; Huang et al., 2016a, 2016b), capsaicin treated injured rats showed greater ERK44/42 and pERK44/42 expression. Pretreatment with BDNF reduced ERK44/42 and pERK44/42 expression after injury, a pattern of results that mirrors our behavioral observations. An ANOVA revealed that the Surgery × BDNF interaction was statistically significant for both ERK44/42 and pERK44/42, all F's > 8.27, $p < 0.01$. Post hoc comparisons confirmed that the sham-operated rats treated with BDNF differed from the sham-operated vehicle treated rats and BDNF treated transected rats ($p < 0.05$).

3.5. Experiment 5: Spinal cord injury transforms how BDNF affects KCC2 expression

GABA function is regulated by two co-transporters, K^+-Cl^- (KCC2) and Na^+-K^+-Cl^- cotransporter 1 (NKCC1), which regulate the outward and inward flow of Cl^-, respectively (Cramer et al., 2008). Early in development, KCC2 levels are low and the outward flow of Cl^- is impeded. As a result, there is a high intracellular concentration of Cl^- (Ben-Ari, 2002). Under these conditions, engaging the $GABA_A$ receptor will allow Cl^- to exit the cell, which has a depolarizing effect. The developmental increase in membrane-bound KCC2 leads to a reduction in intracellular Cl^- levels, establishing the ionic gradient that promotes the inward flow of Cl^- through the $GABA_A$ receptor. This generates the hyperpolarizing (inhibitory) effect associated with GABA transmission.

Recent evidence suggests that BDNF enhances neural excitability within the spinal cord in uninjured subjects because it reduces membrane-bound KCC2, and thereby attenuates GABAergic inhibition (Coull et al., 2005; Lu et al., 2009; Merighi et al., 2008; Rivera et al., 2002). Interestingly, there is some evidence that spinal cord injury may transform how BDNF affects KCC2 (Boulenguez et al., 2010; Shulga et al., 2008). Here we explore this possibility by comparing the effect of BDNF on membrane-bound KCC2 in spinally transected and uninjured (sham-operated) rats.

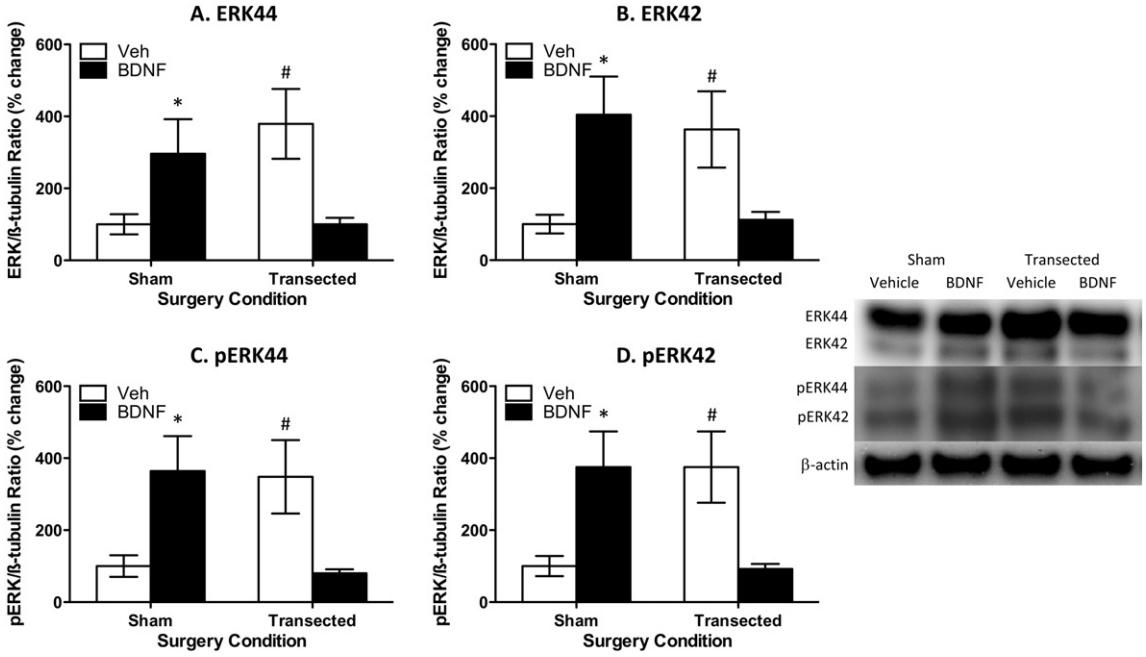


Fig. 5. BDNF increases ERK1/2 and pERK1/2 protein expression in sham-operated rats and attenuates expression after spinal transection. Rats had undergone a spinal transection or sham surgery. The next day, they were given i.t. BDNF (filled) or vehicle (unfilled) followed by capsaicin. Two hours after capsaicin treatment, the lumbar-sacral tissue was collected for protein assays. ERK44 (A), ERK42 (B), pERK44 (C), and pERK42 (D) expression was normalized to β-actin expression and is presented as a percentage change relative to the vehicle-treated sham controls. The error bars indicate the standard error of the mean (SEM). * Indicates that the group differs from both the sham-operated vehicle treated, and transected BDNF treated, groups ($p < 0.05$). # Indicates that the group differs from both the sham-operated vehicle treated, and transected BDNF treated, groups ($p < 0.05$).

The experimental design is illustrated at the top of Fig. 6. Twenty-four rats (n = 6 per condition) were randomly assigned to receive a spinal transection at T2 or sham surgery. Baseline behavioral reactivity was tested using von Frey stimuli 24 h later. After baseline testing, half of the subjects were given an intrathecal injection of BDNF (0.4 μg) and half were administered vehicle. Mechanical reactivity was re-assessed at 0, 1, and 2 h after BDNF injection. Subjects were then euthanized and a one-centimeter section of the spinal cord containing the lumbar enlargement (L3–L5) region was collected. Samples were hemi-dissected into dorsal and ventral halves, and went through fractionation for Western blotting. KCC2 expression was normalized to β-actin expression level, and is presented as a fold change relative to the vehicle group. The protein expression of membrane-bound fraction was then normalized to the cytoplasmic fraction yielding a membrane-bound/cytoplasmic ratio. Cadherin levels, measured with Western blotting, were used to verify fractionation procedure.

Prior to BDNF treatment, sham-operated subjects were more responsive to mechanical stimulation (sham = 5.72 ± 0.06; transected = 6.13 ± 0.04 [mean ± SE]), $F_{(1, 20)} = 71.68$, $p < 0.0001$. BDNF induced an EMR in sham-operated, but not transected, rats (Fig. 6). An ANOVA revealed that the main effect of surgery and drug treatment, as well as Surgery × Drug treatment interaction, were statistically significant (all F values > 15.6, $p < 0.001$). Post hoc comparisons confirmed that sham-operated rats that received BDNF (Sham- > BDNF) differed from the other groups ($p < 0.05$).

To explore the impact of our experimental treatments on the distribution of KCC2, we calculated the ratio of KCC2 associated with the membrane-bound fraction relative to cytoplasmic in the dorsal halves (Fig. 7). In sham-operated rats, BDNF reduced the relative proportion of KCC2 contained within the membrane fraction. In contrast, in transected rats, BDNF increased the expression of membrane-bound KCC2. An ANOVA confirmed that the interaction between Surgery × BDNF treatment was statistically significant ($F_{(1, 20)} = 8.69$, $p < 0.05$).

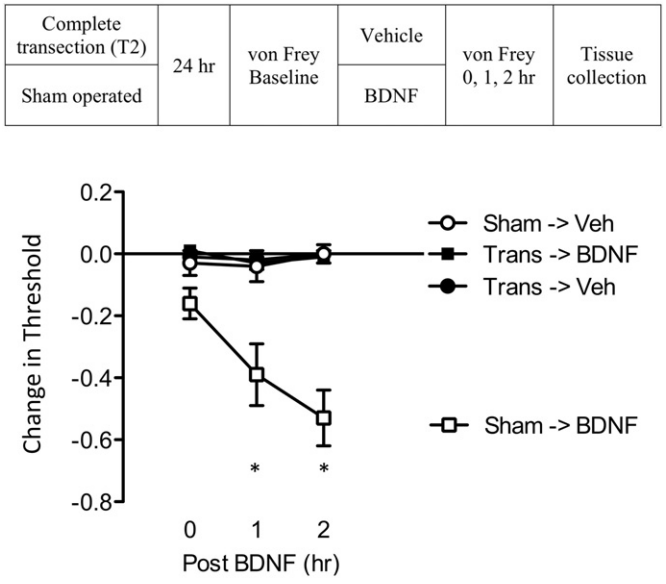


Fig. 6. BDNF induced an EMR in sham-operated rats, but not in transected rats. The experimental design is illustrated at the top. Subjects received either a spinal transection (Trans; circles) or a sham surgery (Sham; squares). The next day, BDNF (filled symbols) or vehicle (unfilled symbols) was administered (i.t.). The y-axis depicts linearized mechanical reactivity scores [log 10 (10,000 g)] as a change from baseline over time after drug treatment (collapsed across leg). The error bars indicate the standard error of the mean (SEM). * Indicates statistical significance ($p < 0.05$).

3.6. Experiment 6: BDNF and spinal cord injury transform how a GABA agonist (muscimol) affects nociceptive sensitization

Replicating others (Coull et al., 2005), we found that BDNF reduces membrane-bound KCC2 in uninjured rats, which should reduce GABAergic inhibition. After spinal cord injury, BDNF had a restorative effect that increased membrane-bound KCC2, which should re-establish GABAergic inhibition. Here, we seek functional evidence that these cellular changes impact how GABA affects behavioral reactivity and the development of capsaicin-induced EMR. We addressed this issue by assessing the effect of the GABA agonist muscimol on behavioral reactivity in BDNF treated injured and uninjured rats. We predicted that muscimol would inhibit behavioral reactivity and capsaicin-induced EMR in uninjured rats, and that pretreatment with BDNF would eliminate this effect. Spinal cord injury should flip how these drug treatments affect behavioral reactivity, eliminating the inhibitory effect of muscimol treatment. In injured rats, pretreatment with BDNF should re-establish GABAergic inhibition and attenuate capsaicin-induced EMR.

The experimental design is illustrated at the top of Fig. 8. Forty-eight subjects (n = 6 per condition) received either a full transection at the second thoracic vertebrae or a sham surgery. Twenty-four hours later, baseline mechanical reactivity was tested using von Frey stimuli. After baseline testing, half of the subjects were given an i.t. injection of BDNF (0.4 μg) and half were administered with vehicle. Mechanical reactivity was re-assessed at 0 and 1 h after BDNF injection. Following the test administered at 1 h after BDNF injection, subjects were given muscimol (i.t.) or its vehicle. Each drug administration was followed by a 20 μL of 0.9% saline flush. Fifteen minutes later, mechanical reactivity was re-assessed. Next, all subjects received a subcutaneous injection of capsaicin (1%). Mechanical reactivity was re-assessed again at 0, 1, and 2 h post capsaicin injection. This yielded a 2 (transected vs. sham-operated) × 2 (BDNF vs. vehicle) × 2 (muscimol vs. vehicle) factorial design.

Replicating the previous result, sham-operated were more responsive to mechanical stimulation (sham = 5.72 ± 0.04; transected = 6.12 ± 0.05 [mean ± SE]; $F_{(1, 40)} = 94.99$, $p < 0.0001$). As expected, BDNF enhanced mechanical reactivity in sham-operated rats (Post BDNF; Fig. 8A). Spinal cord injury eliminated this effect (Fig. 8C). An ANOVA revealed that the main effect of surgery and BDNF, as well as their interactions, were statistically significant (all F values > 73.91, $p < 0.0001$). Post hoc comparisons of the group means showed that, in sham-operated rats, the groups that received BDNF differed from the vehicle treated groups ($p < 0.05$). In contrast, after spinal cord injury, no group comparison was statistically significant ($p > 0.05$).

In sham-operated rats (Fig. 8A), BDNF transformed how muscimol (Post Mus) affected mechanical reactivity, eliminating its inhibitory effect and unveiling an excitatory-like action that fostered EMR. In contrast, after spinal cord injury (Fig. 8C), BDNF appeared to enhance the inhibitory action of muscimol. An ANOVA revealed that the main effect of surgery, BDNF, Surgery × BDNF interaction and Surgery × Muscimol interaction, as well as the Surgery × BDNF × Muscimol three-way interactions were statistically significant (all F values > 6.35, $p < 0.05$). The three-way interaction indicates that the effect of muscimol depends upon *both* spinal cord injury and BDNF treatment. Post hoc comparisons of the group means showed that in sham-operated rats, the BDNF-treated groups differed from the vehicle controls ($p < 0.05$). Further, the group that received BDNF and muscimol (BDNF- > Mus) differed from the group received BDNF alone (BDNF- > Veh). In contrast, after spinal cord injury, no group comparison was statistically significant ($p > 0.05$).

Administration of capsaicin (Post Cap) enhanced the group differences. As expected, capsaicin induced an EMR in sham-operated vehicle-treated rats (Veh- > Veh) and this effect was inhibited by muscimol (Veh- > Mus; Fig. 8A). BDNF-treated rats (BDNF- > Veh) exhibited a stronger EMR and this effect was amplified by muscimol

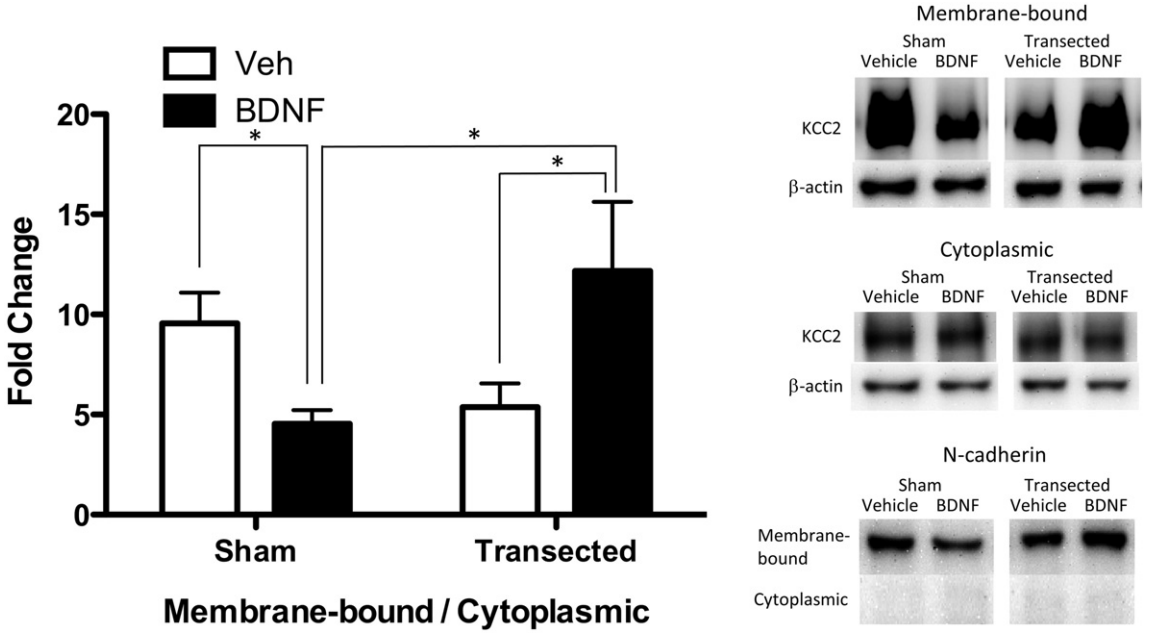


Fig. 7. Spinal cord injury transforms how BDNF affects KCC2 expression. Rats were given i.t. BDNF (filled) or vehicle (unfilled). The y-axis depicts the fold change of membrane-bound/cytoplasmic ratio. The error bars depict ±SEM. * Indicates statistical significance ($p < 0.05$).

treatment (BDNF- > Mus). In contrast, after spinal cord injury muscimol inhibited capsaicin-induced EMR in BDNF treated rats (BDNF- > Mus), and tended to amplify EMR in the absence of BDNF (Veh- > Mus; Fig. 8C). An ANOVA revealed that the main effect of surgery, BDNF, and their interaction were statistically significant. Further, the BDNF × Muscimol and the Surgery × BDNF × Muscimol interactions were statistically significant (all Fs > 11.5, $p < 0.005$). Again, the three-way interaction indicates that the impact of muscimol on EMR depends upon both spinal cord injury and BDNF treatment. Post hoc comparisons of the sham-operated groups showed all group comparisons were significantly different ($p > 0.05$). In transected rats, the groups that received BDNF differed from the vehicle controls ($p < 0.05$).

4. Discussion

Recent studies have shown that BDNF can foster nociceptive sensitization within the spinal cord, a pronociceptive effect that could contribute to the development of chronic pain (Beggs and Salter, 2013; Coull et al., 2005; Garraway et al., 2003; Merighi et al., 2008). Yet, other work suggested that BDNF can quell nociceptive sensitization and promote adaptive plasticity (Boyce and Mendell, 2014; Gomez-Pinilla et al., 2007; Gomez-Pinilla et al., 2001; Huie et al., 2012; Weishaupt et al., 2012). Prior studies from our laboratory supported the latter view, with research demonstrating that behavioral training establishes a state that counters the spinal learning impairment and EMR (maladaptive plasticity) induced by variable intermittent shock or peripheral treatment with the irritant capsaicin (reviewed in Grau et al., 2006; Grau et al., 2012; Grau et al., 2014). Prior work has shown that pretreatment with the BDNF sequestering antibody TrkB-IgG blocks the restoration of spinal learning (Baumbauer et al., 2009; Huie et al., 2012), which suggests that BDNF plays an essential role. In the present paper, we asked whether BDNF also mediates the effect of training on spinally-mediated nociceptive sensitization. We addressed this issue by exposing spinally transected rats to an extended series of fixed spaced stimulation. As previously reported (Baumbauer et al., 2012), this training attenuated the development of capsaicin-induced EMR. This effect of training was blocked by i.t. TrkB-IgG.

Having established that activation of the TrkB receptor plays an essential role, we then asked whether pretreatment with BDNF could substitute for training and attenuate capsaicin-induced EMR. We found that the BDNF, per se, had little effect on mechanical reactivity in spinally transected rats and that the drug blocked the development of capsaicin-induced EMR. Elsewhere we have shown that BDNF also blocks the development of shock-induced EMR (Huie et al., 2012). We then asked whether BDNF can reverse the acute effect of capsaicin treatment. We found that BDNF given 1 h after the application of capsaicin had no effect, which suggests that BDNF (at the dose tested) does not impact the maintenance of nociceptive sensitization after it has been induced. While BDNF did not reverse the acute effect of capsaicin treatment, prior work suggests that it can mitigate the long-term consequences of nociceptive sensitization, restoring the capacity to learn when subjects are tested 24 h after noxious stimulation (Huie et al., 2012).

Based on prior work (Boulenguez et al., 2010; Shulga et al., 2008), we hypothesized that spinal cord injury transforms how BDNF affects nociceptive processing within the spinal cord. In the absence of injury, BDNF generally promotes nociceptive sensitization (Coull et al., 2005; Garraway et al., 2003) whereas after SCI BDNF may quell neural excitability (Boulenguez et al., 2010). We evaluated these predictions by comparing the effect of BDNF in sham-operated and spinally transected rats. We found that BDNF enhanced mechanical reactivity and capsaicin-induced EMR in sham-operated rats. After SCI, BDNF attenuated spinally-mediated nociceptive sensitization. To reinforce our behavioral observations, we assessed ERK and pERK expression within the dorsal horn. These cellular indices of central sensitization yielded a pattern of results that mirrored our behavioral data; in intact rats, BDNF enhanced ERK/pERK expression, whereas in transected rats it had the opposite effect. Interestingly, we also observed that ERK/pERK expression was generally enhanced in the vehicle treated transected rats (relative to the vehicle treated sham controls). This cellular effect is consistent with other work demonstrating that descending pathways modulate nociceptive circuits within the spinal cord and generally inhibit neural excitability (Crown and Grau, 2005; Garraway and Hochman, 2001; Gjerstad et al., 2001; Sandkuhler and Liu, 1998). SCI would remove this inhibitory influence, leading to an increase in ERK/pERK expression. Against this sensitized background, BDNF lowers rather than enhances ERK/pERK expression.

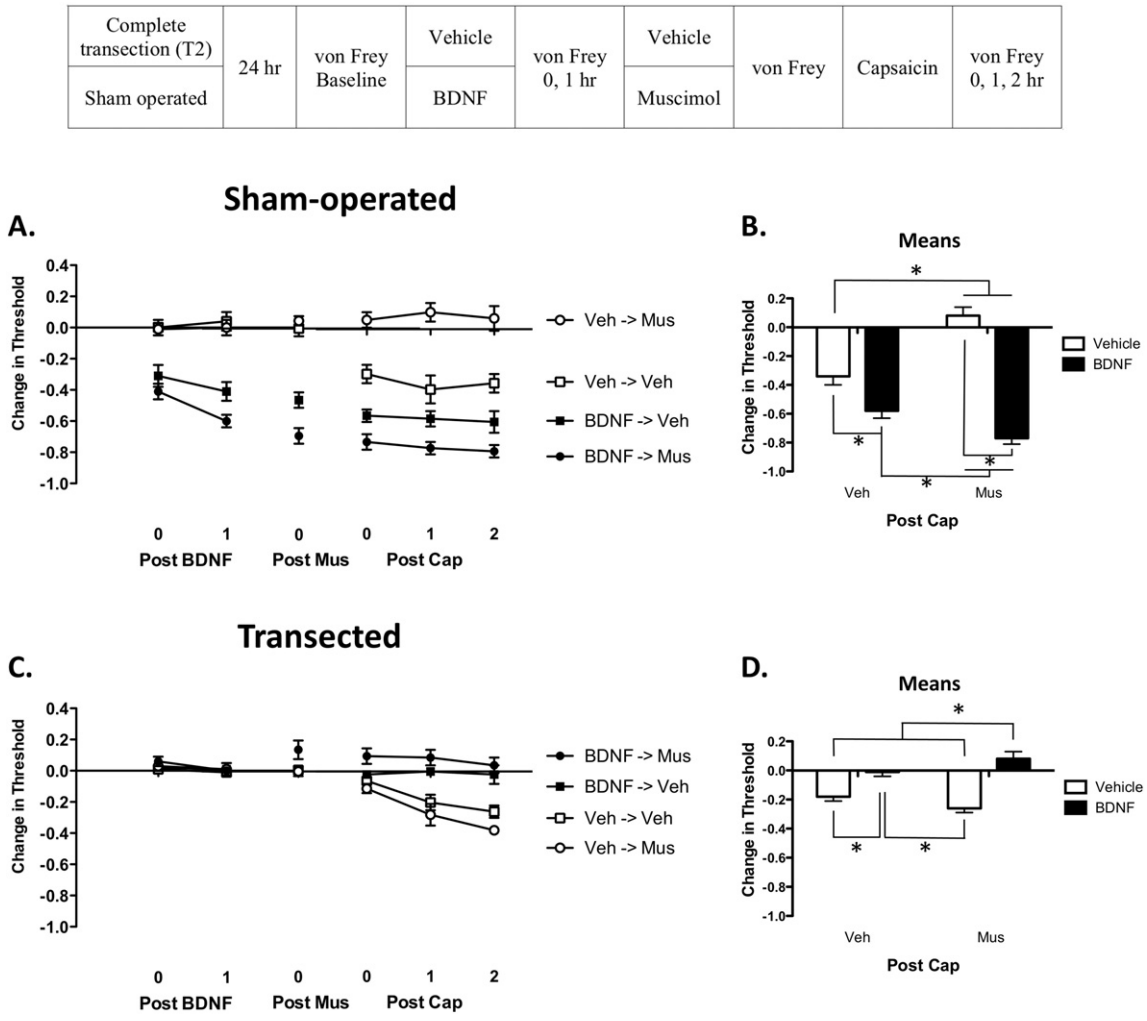


Fig. 8. BDNF and spinal cord injury transform how a GABA agonist (muscimol) affects nociceptive sensitization. The experimental design is illustrated at the top. Subjects received either a sham surgery (A) or a spinal transection (C). The next day, BDNF (filled symbols) or vehicle (unfilled symbols) was administered (i.t.). One hr. after BDNF treatment, muscimol (circles) or vehicle (squares) was administered (i.t.) followed by capsaicin. The y-axis depicts linearized mechanical reactivity scores [log 10 (10,000 g)] as a change from baseline (collapsed across leg). Mechanical reactivity was assessed after treatment with BDNF (Post BDNF), muscimol (Post Mus), and capsaicin (Post Cap). Mean reactivity (collapsed across time and leg) post capsaicin in sham-operated (B) and spinally transected (D) rats. The error bars indicate the standard error of the mean (SEM). * Indicates statistical significance ($p < 0.05$).

BDNF is thought to foster the development of nociceptive sensitization in uninjured animals by decreasing membrane-bound KCC2, which would increase the intracellular concentration of Cl^-. Under these conditions, engaging the $GABA_A$ receptor would allow Cl^- to exit the cell, which would have a depolarizing (rather than a hyperpolarizing) effect. As reported by others (Boulenguez et al., 2010; Coull et al., 2005), we found that BDNF down-regulated membrane-bound KCC2 in uninjured rats, which would lower GABAergic inhibition. SCI per se also lowered KCC2 expression and this effect was reversed by BDNF. To evaluate whether these changes in KCC2 expression have functional consequences, we assessed the behavioral effect of the $GABA_A$ agonist muscimol. Muscimol inhibited mechanical reactivity in intact, but not transected, rats. In uninjured rats, muscimol also inhibited the development of capsaicin-induced EMR and this effect was eliminated by BDNF. Conversely, in transected rats muscimol alone had little effect on spinally-mediated nociceptive sensitization, but inhibited capsaicin-induced EMR in BDNF treated rats. The results imply that BDNF has opposing effects on GABAergic inhibition in intact, and injured rats; in intact rats, BDNF lessens GABAergic inhibition whereas after SCI, BDNF acts to restore the inhibitory effect of GABA agonists.

In another recent paper (Huang et al., 2016a, 2016b), we explored the injury-induced transformation of GABAergic inhibition in more detail. As reported by others (Sivilotti and Woolf, 1994; Sorkin et al., 1998; Zhang et al., 2001; Baba et al., 2003; Dougherty and Hochman, 2008), the $GABA_A$ antagonist bicuculline enhanced nociceptive reactivity in uninjured rats, implying that GABA normally inhibits neural excitability. SCI eliminated bicuculline-induced nociceptive sensitization and unveiled a paradoxical effect wherein pretreatment with the $GABA_A$ antagonist blocked the development of capsaicin-induced EMR (Huang et al., 2016a, 2016b). This suggests that, after SCI, GABA plays an essential role in the development of spinally-mediated nociceptive sensitization. We related this transformation to an injury-induced down-regulation of membrane-bound KCC2 and showed that blocking this channel in uninjured rats emulated the effect of spinal transection. Conversely, blocking the Na+-K+-Cl cotransporter 1 (NKCC1) channel with bumetanide (which should lower intracellular Cl^- concentrations) in transected rats reversed the effect of bicuculline, re-instating a pattern of results indicative of GABAergic inhibition. Beyond these acute effects, observed within 48 h of SCI, others have shown that SCI induces a lasting reduction in membrane-bound KCC2 that is evident weeks after injury (Hasbargen et al., 2010; Kaila et al., 2014). The resultant reduction in GABAergic inhibition has been linked to the development of chronic pain and spasticity (Gwak and Hulsebosch, 2011). A KCC2-dependent reduction in GABAergic inhibition also contributes to the development of hyperalgesia in diabetic rats (Jolivalt et al., 2008), showing that this loss of inhibition occurs in a variety of chronic pain models.

Taken together, our results imply that spinal cord injury transforms how GABAergic processes regulate nociceptive processing. Injury may have this effect by disrupting descending serotonergic (5HT) fibers. Supporting this, we have shown that blocking serotonergic transmission in intact rats, through i.t. application of a 5HT-1A antagonist, has the same effect as spinal transection (Huang et al., 2015). Conversely, after SCI, i.t. application of a 5HT-1A agonist appears to reinstate GABAergic inhibition. Evidence suggests that the essential serotonergic fibers descend through the dorsolateral funiculus (DLF), because lesions limited to this region have the same effect as a complete transection (Crown and Grau, 2005).

Our results reinforce prior studies demonstrating that BDNF can promote adaptive plasticity after SCI and contributes to the beneficial effects of training/exercise (Boyce and Mendell, 2014; Gomez-Pinilla and Vaynman, 2005; Gomez-Pinilla et al., 2001; Huie et al., 2012; Hutchinson et al., 2004). Our work goes beyond these studies to show that BDNF can counter the development of spinally-mediated nociceptive sensitization after SCI and that this effect is related to increased KCC2 expression and the reinstatement of GABAergic inhibition. Our findings are also consistent with prior work implicating the development of chronic pain after SCI to a reduction in GABAergic inhibition and the down-regulation of KCC2 (Coull et al., 2005; Medina et al., 2014; Merighi et al., 2008; Smith, 2014). An injury-induced reduction in GABAergic inhibition is also implicated in the development of spasticity and this effect too has been linked to a reduction in KCC2 (Boulenguez et al., 2010). Because BDNF up-regulates KCC2 after neural injury (Shulga et al., 2008), and attenuates spasticity (Boulenguez et al., 2010), there was reason to expect it would also reduce nociceptive sensitization (Huie et al., 2012). However, this prediction appeared in conflict with a much larger literature demonstrating that BDNF contributes to the development of nociceptive sensitization (Beggs and Salter, 2013; Merighi et al., 2008; Smith, 2014). We addressed these contradictory observations and showed that SCI flips how BDNF affects KCC2, GABAergic inhibition, and the development of nociceptive sensitization. BDNF also has opposing effects on rate-dependent depression (a behavioral index of GABAergic inhibition; Lee-Kubli and Calcutt, 2014) and this effect too has been tied to a switch in how BDNF affects KCC2 (Boulenguez et al., 2010).

At a functional level, an injury-induced modification in BDNF/GABA function parallels the natural transformation that occurs during development. Early in development, KCC2 levels are low and GABA has a depolarizing effect (Fiumelli and Woodin, 2007; Kaila et al., 2014). In this state, BDNF promotes neuronal growth and adaptive plasticity. As the system matures, an increase in KCC2 leads to a reduction in intracellular Cl^- concentration within neurons, which causes GABA to have an inhibitory effect (Ferrini and De Koninck, 2013; Medina et al., 2014). This developmental shift is accompanied by a modification in how BDNF affects spinal function, causing it to bring about a reduction in membrane-bound KCC2 and GABAergic inhibition (Boulenguez et al., 2010; Rivera et al., 2002; Rivera et al., 2005; Zhang et al., 2013). By reducing GABAergic inhibition, BDNF recapitulates an earlier developmental state wherein it acts to amplify neural transmission and encourage activity-dependent plasticity. While this would serve to conserve metabolic resources (by removing the GABAergic brake on neural transmission; Kaila et al., 2014; Rivera et al., 2005), it can foster the development of maladaptive plasticity (Beggs and Salter, 2013; Merighi et al., 2008). Interestingly, neural injury also appears to push the system towards a state akin to early development, with a reduction in KCC2 and GABA-mediated inhibition (Boulenguez et al., 2010; Shulga et al., 2008). After the transformation in GABA function occurs, there is a reversal in how BDNF affects KCC2. Interestingly, this reversal in BDNF's action appears linked to the alteration in GABAergic activity, because it is not observed if the hyperpolarizing shift is blocked after injury (Shulga et al., 2008). This observation suggests that the effect of BDNF on cellular function is regulated by the current state of GABAergic inhibition, shifting the system towards hyperpolarization (by down-regulating KCC2) when inhibition is strong and acting to restore inhibition (by up-regulating KCC2) when it is weak. In this way, BDNF appears to be have a homeostatic effect (Wenner, 2014).

While it is clear that spinal cord injury can transform BDNF function, the mechanisms that mediate this transformation remains opaque. One intriguing possibility links the alteration in BDNF's effect on KCC2 expression to phospholipase C-γ (PLC-γ), an effector of TrkB signaling. Engaging the TrkB receptor also activates Shc, which is involved in the regulation of KCC2 (Rivera et al., 2004). Interestingly, a BDNF-induced increase in Shc appears to down-regulate KCC2 when PLC-γ is present, but up-regulate KCC2 when PLC-γ is absent. This observation has led researchers to suggest that the developmental shift in GABAergic function may be linked to increased expression of PLC-γ (Rivera et al., 2005; Rivera et al., 2004). Further, recent work suggests that PLC-γ is down-regulated by SCI (Tashiro et al., 2015), which could transform how BDNF affects KCC2. While intriguing, additional observations suggest that other factors must be considered. For example, evidence suggests that the development of maladaptive plasticity after SCI is tied to mGluR1 transmission (Ferguson et al., 2008), which is thought to impact cellular plasticity through PLC-γ (Abraham, 2008). Another important consideration concerns the impact of neural excitation on non-neuronal cells and the source of BDNF. In intact animals, it has been suggested that nociceptive excitation leads to the release of ATP, which engages microglia that release BDNF and foster maladaptive plasticity through a reduction in GABAergic inhibition (Coull et al., 2005). Microglia also appear to play a role in the development of maladaptive plasticity after SCI, but here the increase in neural excitability has been tied to the release of tumor necrosis factor (TNF) and the trafficking of calcium-permeable AMPA receptors to the active zone of the synapse (Huie et al., 2012; Huie et al., 2015). In the injured state, BDNF seems to quell this over-excitation, a finding that is consistent with the idea that BDNF and TNF can act in concert to regulate homeostatic scaling (Stellwagen and Malenka, 2006; Turrigiano, 2006). It should also be recognized that the development of maladaptive plasticity after SCI is associated with alterations that would be expected to increase the intracellular concentration of Ca^{2+} (Ferguson et al., 2008; Ferguson et al., 2006; Huie et al., 2015) and that this factor too may influence how BDNF affects cellular function. BDNF, an increase in GABAergic inhibition, antagonizing the NMDAR or mGluR1, or engaging the 5HT-1A receptor may attenuate maladaptive plasticity after SCI because these treatments have a common effect—they act to lower intracellular concentrations of Ca^{2+} and thereby dampen neural excitability (Crown and Grau, 2001; Ferguson et al., 2008; Ferguson et al., 2006). Further studies are needed to explore these potential mechanisms.

Recognizing that how BDNF affects spinal function depends upon the presence of descending fibers, and the cellular context, can help us to understand why this ligand has varied effects on recovery and the circumstances under which treatment will yield a beneficial versus adverse effect. In general, BDNF appears to promote adaptive plasticity when communication with the brain is disrupted by a full transection (Gomez-Pinilla et al., 2007; Grau et al., 2012; Grau et al., 2014; Huie et al., 2012). Further, the beneficial effects of training/exercise after SCI seem to depend upon BDNF release (Boyce and Mendell, 2014; Gomez-Pinilla and Vaynman, 2005; Hutchinson et al., 2004). BDNF may generally promote function after complete transection because it dampens over-excitation and fosters activity-dependent plasticity within selective neural circuits. However, if descending fibers are preserved, the application and/or up-regulation of BDNF could potentially encourage the development of maladaptive plasticity, promoting over-excitation and strengthening circuits that contribute to the maintenance of chronic pain (Coull et al., 2005; Merighi et al., 2008). Further, by promoting the diffuse saturation of NMDAR-mediated plasticity, the capacity to adapt to new environmental relations would be compromised (Ferguson et al., 2008; Grau et al., 2014). The implication is that the clinical usefulness of BDNF as a treatment will depend upon the mode of expression, extent of injury and degree of recovery.

Conflict of interest

The authors declare no competing financial interests.

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