

# Blockade of transient receptor potential cation channel subfamily V member 1 promotes regeneration after sciatic nerve injury

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## Abstract

The transient receptor potential cation channel subfamily V member 1 (TRPV1) provides the sensation of pain (nociception). However, it remains unknown whether TRPV1 is activated after peripheral nerve injury, or whether activation of TRPV1 affects neural regeneration. In the present study, we established rat models of unilateral sciatic nerve crush injury, with or without pretreatment with AMG517 (300 mg/kg), a TRPV1 antagonist, injected subcutaneously into the ipsilateral paw 60 minutes before injury. At 1 and 2 weeks after injury, we performed immunofluorescence staining of the sciatic nerve at the center of injury, at 0.3 cm proximal and distal to the injury site, and in the dorsal root ganglia. Our results showed that Wallerian degeneration occurred distal to the injury site, and neurite outgrowth and Schwann cell regeneration occurred proximal to the injury. The number of regenerating myelinated and unmyelinated nerve clusters was greater in the AMG517-pretreated rats than in the vehicle-treated group, most notably 2 weeks after injury. TRPV1 expression in the injured sciatic nerve and ipsilateral dorsal root ganglia was markedly greater than on the contralateral side. Pretreatment with AMG517 blocked this effect. These data indicate that TRPV1 is activated or overexpressed after sciatic nerve crush injury, and that blockade of TRPV1 may accelerate regeneration of the injured sciatic nerve.

**Key Words:** nerve regeneration; peripheral nerve regeneration; transient receptor potential cation channel subfamily V member 1; capsaicin receptor; vanilloid receptor; TRPV1 antagonist; nociceptor; nerve crush injury; Wallerian degeneration; axon; NSFC grant; neurites; neural regeneration

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## Introduction

The structure and function of transient receptor potential cation channel subfamily V member 1 (TRPV1) have been studied extensively since the receptor was first cloned in 1977. TRPV1 is a nociceptor and is mainly expressed in peripheral nerve endings, as well as throughout the central nervous system (Chen et al., 2015; Naziroğlu and Ovey, 2015; Saffarzadeh et al., 2015; Wang et al., 2015). In the central processes of dorsal root ganglion (DRG) neurons, the TRPV1 receptor plays an important role in the integration of painful stimuli and impairments in conduction (St Pierre et al., 2009; Jeong and Seong, 2014; Malek et al., 2015; Wang et al., 2015). It is activated by capsaicin and vanillin, and can be altered by regional physical and chemical changes, such as temperatures over 42°C, pH changes below 6.0, and endogenous lipid materials (Kaszas et al., 2012; Jeong and Seong, 2014; Morales-Lázaro et al., 2014; Islas et al., 2015; Horváth et al., 2015). The receptor can also be blocked by a number of bioactive substances, such as capsazepine (a competitive

capsaicin antagonist), ruthenium red (a noncompetitive antagonist), resiniferatoxin, LJO-328, SC0030, linopirdine, and AMG517 (Johansen et al., 2006; Cahusac, 2009; Mitchell et al., 2010; Artim et al., 2011; Calahan et al., 2013; Heng et al., 2014). AMG517 is a potent specific antagonist at TRPV1, and has been used in the clinic as a treatment for chronic pain (Kort and Kym, 2012).

As a membrane protein, TRPV1 interacts structurally and functionally with other membrane protein/receptor molecules. For example, its interaction with the angiotensin II receptor affects neural regeneration (Anand et al., 2013). Additionally, TRPV1 expression is colocalized with the nerve growth factor receptor TrkA (Zheng et al., 2015). Therefore, the TRPV1 intracellular signaling pathway is not only limited to pain transmission, but also regulates a variety of other functions. Few studies have investigated whether regional TRPV1 expression is enhanced after nerve injury, or whether alterations in TRPV1 function affect neural regeneration; therefore, to explore the relationship between TRPV1 and

nerve injury, we used a rat model of sciatic nerve crush injury to examine TRPV1 expression and the effect of blocking TRPV1 function on axonal regeneration.

## Materials and Methods

### Experimental animals

A total of 40 healthy male Sprague-Dawley rats weighing 240–260 g were purchased from the Experimental Animal Center of Shanxi Medical University, China (license No. SCXK (Jin) 2009-0001). All procedures were approved by the Shanxi Medical University Animal Ethics Committee in China. Rats were housed at room temperature under a 12-hour light/dark cycle and allowed free access to food and water.

### Modeling sciatic nerve crush injury

Sciatic nerve crush injury was performed using a modification of the sural nerve crush method described by Li et al. (2004). In brief, the rats were anesthetized with 1% sodium pentobarbital (4 mL/kg intraperitoneally), placed on their right side, and the hindlimbs stretched and fixed in position. The skin was shaved and cleaned with povidone iodine. A 1–2-cm incision was made lateral to the right knee, and the muscle was isolated. The sciatic nerve was dissociated between the biceps femoris and gluteus maximus, and clamped for 1 minute, approximately 0.5 cm proximal to the common peroneal nerve bifurcation, to cause crush injury (Figure 1). The muscle and skin were sutured, and the same procedure was carried out in the left (control) hindlimb, except the sciatic nerve was dissociated but not crushed. The rats were allowed to recover in a clean, warm, dry environment. Both motor and sensory dysfunction was evident following crush injury.

### AMG517 administration

The TRPV1 antagonist AMG517 (Selleckchem, Houston, TX, USA) was dissolved to 0.25% in 80% ethanol/20% dimethyl sulfoxide, divided into aliquots, and stored at –20°C until use (Wanner et al., 2012).

The rats were randomized into four groups of 10. In the 1-week injury + vehicle group, rats underwent right sciatic nerve crush injury and tissue was collected and observed 1 week after injury. In the 2-week injury + vehicle group, rats underwent right sciatic nerve crush injury, and tissue was collected and observed 2 weeks after injury. In the 1-week injury + AMG517 and 2-week injury + AMG517 groups, AMG517 (300 µg/kg) was injected subcutaneously into the paw ipsilateral to the injury, 60 minutes prior to nerve crush. Meanwhile, only vehicle was injected into paw in 1-week injury and 2-week injury + vehicle groups. Tissue was collected and observed 1 and 2 weeks after injury, respectively.

### Axon counting

The rats were anesthetized with 1% sodium pentobarbital (4 mL/kg intraperitoneally) 1 or 2 weeks after model induction, and the sciatic nerves were obtained bilaterally, comprising the injury site (clamped region, approximately 0.3 cm) and 0.3 cm of tissue proximal and distal to the center of injury. The tissue was fixed in 2.5% glutaraldehyde for 2 hours, treated with sodium cacodylate buffer, and stored at 4°C

until use. For electron microscopy, the tissue was sliced into semithin sections and stained with toluidine blue as follows: samples were washed with sodium dimethylarsenate, postfixed in 1% osmium tetroxide and dehydrated through a graded alcohol series. They were then embedded in Epon-812 epoxy resin, polymerized in an oven at 37–60°C for 3 days until polymerization was complete, and cut into 1-mm sections using an ultramicrotome (EM UC6; Leica Microsystems GmbH, Wetzlar, Germany) with a glasscutter.

For light microscopy, sections were air dried at room temperature for approximately 1 hour, stained with 0.1% toluidine blue for several minutes, air-dried again, permeabilized in xylene, mounted with resin, and observed under a light microscope (BX43; Olympus, Tokyo, Japan). Six fields were randomly selected in each section and photographed under 100× magnification. Myelinated and unmyelinated nerve clusters were counted.

### Immunofluorescence staining for TRPV1

Sciatic nerves and L<sub>4</sub> DRGs were collected bilaterally 1 or 2 weeks after injury, embedded in Optimal Cutting Temperature compound, and stored at –80°C until use. A freezing microtome was used to cut the DRGs and sciatic nerves into 10-µm transverse and longitudinal sections, respectively, to obtain the maximum cross-sectional area. Five to six sections were collected per tissue sample. Sections were placed on polylysine-coated slides, air-dried, and fixed in 4% paraformaldehyde (pH 7.4) at 4°C overnight. Following washes with PBS, the sections were treated with 0.1% Triton-X100, blocked with normal donkey serum, and incubated with goat anti-TRPV1 polyclonal antibody (1:500; Santa Cruz Biotechnology, Dallas, TX, USA) overnight at 4°C, and Alexa Fluoro-488-labeled donkey anti-goat IgG (1:500; Life Technologies, Shanghai, China) in the dark for 1 hour at room temperature. The sections were then mounted with Antifade mountant with DAPI (Life Technologies), and observed and photographed under a fluorescence microscope (BX43; Olympus). To compare the difference in sciatic nerve TRPV1 immunoreactivity between the injured and control sides, fluorescence intensity was converted to average gray value using Image J software (<http://imagej.nih.gov/ij/>). The numbers of TRPV1-positive cells in the ipsilateral and contralateral L<sub>4</sub> DRGs were compared by calculating the percentage of DAPI-positive cells that showed TRPV1 immunoreactivity in five to six sections of DRG.

### Statistical analysis

Data are expressed as the mean ± SEM, and were analyzed using GraphPad software (Prism 5, GraphPad Software, Inc., CA, USA). Intergroup differences were compared by one-way and two-way analysis of variance. *P* < 0.05 was considered statistically significant.

## Results

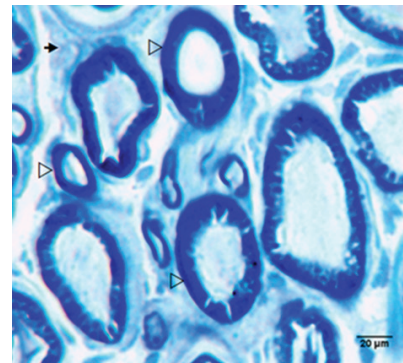
### Nerve regeneration after sciatic nerve crush injury

Under the light microscope, toluidine blue staining in the contralateral nerve revealed a large number of myelinated neurons; axons were surrounded by a dense lamellar myelin

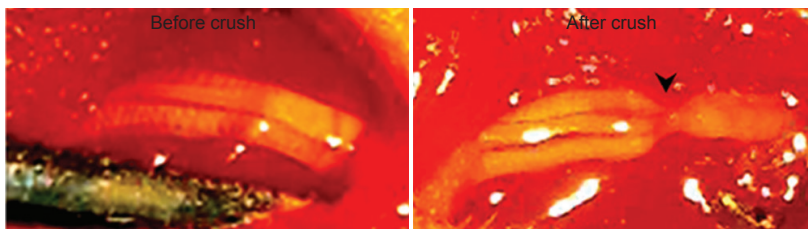
sheath, and the structure was well-defined. Unmyelinated nerve fibers were small in diameter; in most of these, the foci with 2–4 unmyelinated axons were observed against a clear background, showing typical cross-sections of peripheral nerve fibers (Figure 2).

One week after injury, macroscopic examination revealed congestion and edema around the nerve, at the center of the injury site, and in the surrounding tissue. At 2 weeks, the nerve was thickened at the center of the injury site, with congestion and edema still evident. Rats injected with AMG517 before injury showed less congestion and edema and better recovery than vehicle-pretreated animals.

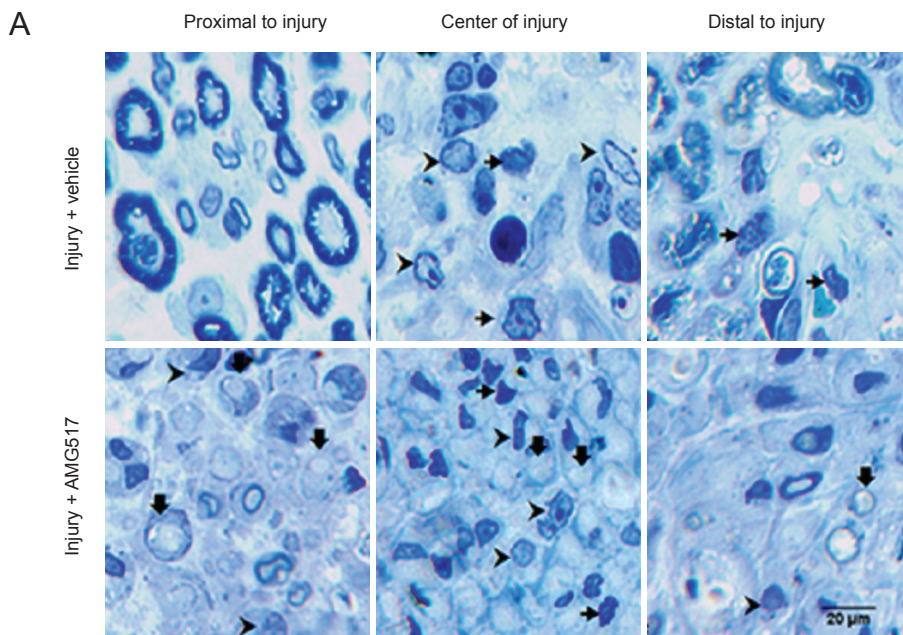
Toluidine blue staining of semithin sciatic nerve sections revealed that, 1 week after injury, few axons and little myelin sheath remained at the injury site and in the region distal to it (Figure 3A). A large number of phagocytic cells



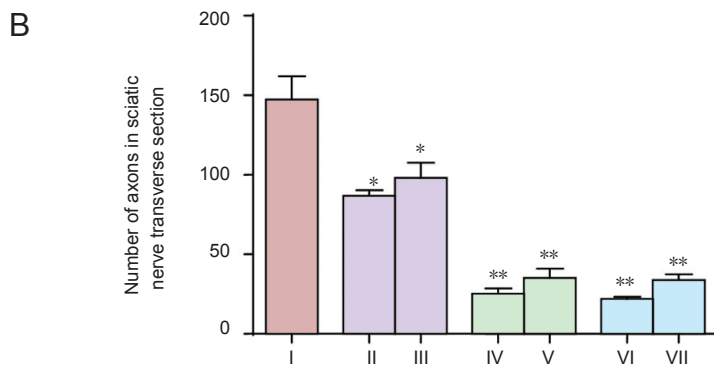
**Figure 2 Morphology of semithin (1-mm) transverse section of normal sciatic nerve (toluidine blue staining).** Scale bar: 20 μm; ►: unmyelinated fiber cluster; ▷: myelinated nerve fiber.

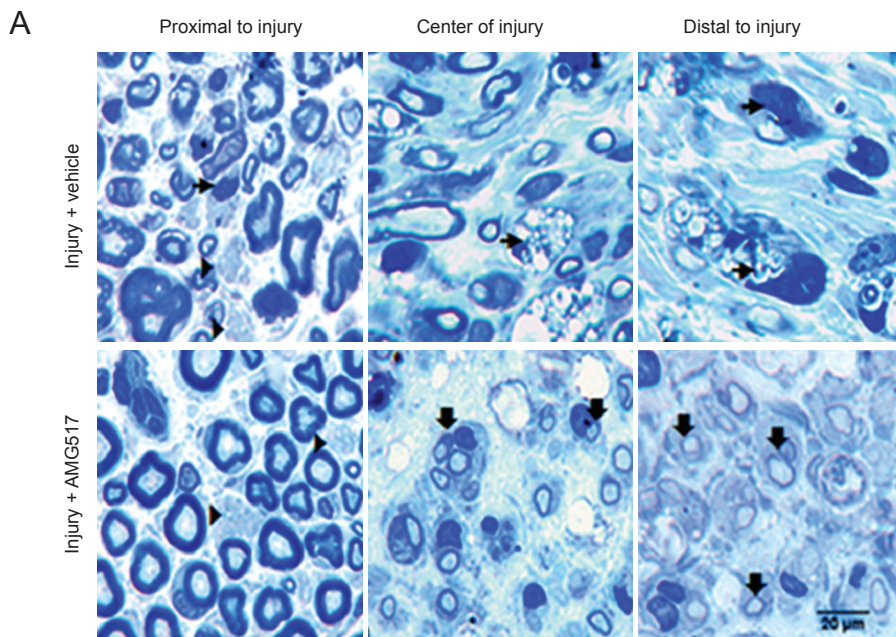


**Figure 1 Operative field in rats before and after sciatic nerve crush injury.** Note the marked narrowing of the nerve at the site of injury (▼).



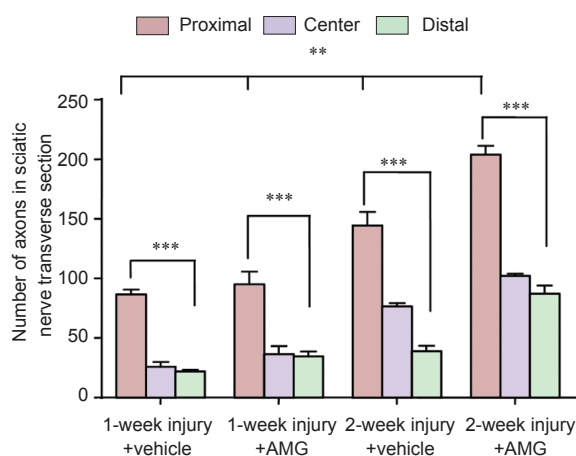
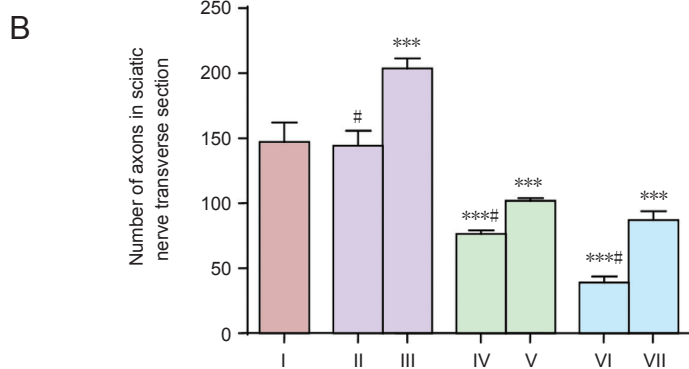
**Figure 3 Effects of AMG517, a TRPV1 antagonist, pretreatment on number of axons in the sciatic nerve 1 week after crush injury (toluidine blue staining).** (A) Sciatic nerve cross-section at three sites around the injury. Proximal to the injury site, axons were sparsely distributed, with wide gaps between them. At the central injury site, there were also few axons, and mononuclear phagocytic cell infiltration and Schwann cell proliferation were visible. Distal to the injury site, marked myelin sheath disintegration and mononuclear phagocytic cell infiltration were observed. In rats pretreated with AMG517, Schwann cell proliferation and new myelin sheath formation were noted in particular at the central injury site, as well as proximal and distal to it. Scale bar: 20 μm; ►: Schwann cells; ►: mononuclear phagocytic cells; ⚡: new myelin sheath. (B) Significantly fewer axons were observed throughout the injury site (proximal, central, distal regions) and in both injury groups (treated, untreated) than in uninjured sciatic nerve; however, more axons were observed after AMG517 pretreatment. \* $P < 0.05$ , \*\* $P < 0.01$ , vs. uninjured side (mean ± SEM,  $n = 10$ ; one-way analysis of variance and the least significant difference test); 1wk: 1 week; P: site proximal to injury; C: center of injury; D: site distal to injury; AMG: AMG517. I: Contralateral; II: 1 wk-P + vehicle; III: 1 wk-P-AMG; IV: 1 wk-C + vehicle; V: 1 wk-c-AMG; VI: 1 wk-D + vehicle; VII: 1 wk-D-AMG.





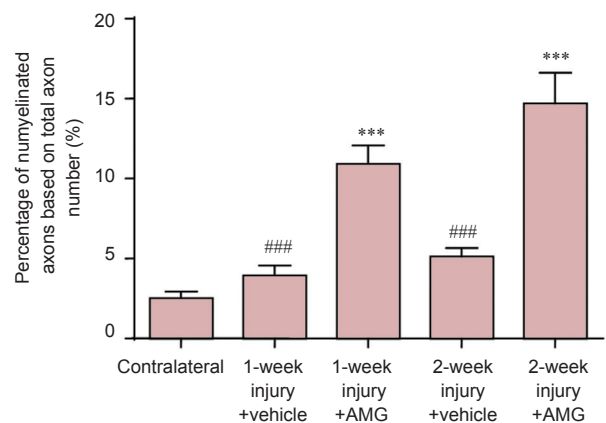
**Figure 4 Effects of AMG517, a TRPV1 antagonist, pretreatment on number of axons in the sciatic nerve 2 weeks after crush injury (toluidine blue staining).**

(A) Sciatic nerve cross-section at three sites around the injury. By 2 weeks, axons had recovered to numbers observed on the uninjured side. Three to five clusters of unmyelinated nerve fibers were observed between myelinated axons. Mononuclear phagocyte infiltration was visible, and myelin disintegration products were cleared by phagocytic cells. In the group pretreated with AMG517, the sciatic nerve cross-section appeared normal at the site proximal to the injury, indicating full recovery. The number of neurites was greater in the region proximal to the injury site than in uninjured nerve. Large clusters (>10) of unmyelinated nerves were visible in all regions. There were noticeably more axons at the central and distal injury sites in the injury + AMG517 group than in the non-pretreated injury group, with widespread formation of new myelin. (B) There were significantly fewer axons at all sites around the injury than on the control side in the non-pretreated injury group, but more axons in the AMG517-pretreated group than in the non-pretreated group. Scale bar: 20  $\mu$ m;  $\blacktriangleright$ : mononuclear phagocytic cells;  $\blacktriangledown$ : new myelin sheath;  $\blacktriangle$ : unmyelinated nerve clusters;  $***P < 0.001$ , vs. contralateral;  $\#P < 0.05$ , vs. corresponding AMG517 group (mean  $\pm$  SEM,  $n = 10$ ; one-way analysis of variance and least significant difference test); 2 wk: 2 weeks; P: proximal site; C: center of injury; D: distal site; AMG: AMG517. I: Contralateral; II: 2 wk-P + vehicle; III: 2 wk-P-AMG; IV: 2wk-C + vehicle; V: 2 wk-C-AMG; VI: 2 wk-D + vehicle; VII: 2 wk-D-AMG.



**Figure 5 Number of axons in sciatic nerve transverse section 1 and 2 weeks after crush injury, with or without AMG517, a TRPV1 antagonist, pretreatment.**

$***P < 0.01$ , 1 vs. 2 weeks, with AMG517 pretreatment;  $***P < 0.001$ , central vs. distal injury site (one-way analysis of variance and the least significant difference test). Data are expressed as the mean  $\pm$  SD;  $n = 10$ . AMG: AMG517.



**Figure 6 Effects of AMG517, a TRPV1 antagonist, pretreatment on regeneration of unmyelinated nerve fibers in the proximal injury site.**

AMG517 promoted the regeneration of unmyelinated axons. In particular, the number of unmyelinated nerve clusters increased markedly 2 weeks post injury.  $###P < 0.001$ , vs. uninjured side;  $***P < 0.001$ , vs. injury + AMG517 groups (mean  $\pm$  SD,  $n = 10$ ; one-way analysis of variance and the least significant difference test); AMG: AMG517.

had infiltrated into the region, and the number of cells at the center of the injury site was markedly elevated. Schwann cell nuclei were large, with distinct nucleoli. The histological characteristics of neurites were absent. Schwann cells had proliferated and surrounded regenerating axons in the tissue proximal to the injury site. Compared with the contralateral nerve, fewer neurites were observed across all three sites. In rats pretreated with AMG517, notable Schwann cell proliferation was seen, and the number of axons was elevated. There was no significant difference in axon number between the injury with vehicle and injury + AMG517 groups in any of the three regions ( $P > 0.05$ ); however, a trend for AMG517 pretreatment to accelerate axon regeneration was observed (**Figure 3B**).

Two weeks after injury, regeneration was evident. Compared with the contralateral side, the number of axons was notably elevated in the region proximal to the injury site, and the number of cells was also greater in the center of the injury site and distal to it. Numerous new myelinated axons surrounded by Schwann cells were visible. These changes, indicative of regeneration, were particularly prominent in rats pretreated with AMG517, at the proximal to the injury site, more neurites were observed than in control nerves; there were more fine, focal, unmyelinated nerve clusters (aggregates of more than five unmyelinated fibers) in the AMG517-pretreated injured nerves than in contralateral or vehicle-pretreated injured nerves ( $P < 0.001$ ), exceeding 10 fibers in most clusters (**Figure 4**). Schwann cells proliferated noticeably at, and distal to, the injury site. Single or multiple new myelin sheaths with thin walls were found in Schwann cells. There were fewer phagocytic cells within foci in the AMG 517-pretreated injury groups than the vehicle-pretreated injury groups, but phagocytosis was still evident (**Figure 4**). Furthermore, in the AMG517-pretreated group, the total number of axons was greater 2 weeks after injury than at 1 week with vehicle-pretreatment ( $P < 0.01$ ; **Figure 5**).

These results indicate that injection of AMG517 in the ipsilateral paw 60 minutes before injury promotes nerve regeneration. The number of axons was noticeably greater at the injury site and around it, especially in unmyelinated nerves in the injury + AMG517 groups (**Figure 6**). Our results confirm that AMG517 blocked TRPV1 and contributed to nerve regeneration.

#### Alterations in TRPV1 expression following sciatic nerve crush injury

Indirect immunofluorescence staining for TRPV1 was negative in longitudinal sections of normal sciatic nerve. However, positive TRPV1 staining was observed at the injury site 1 and 2 weeks after nerve crush. Positive fibers were scattered around the sciatic nerve, mainly near the epineurium. Compared with the vehicle-pretreated injury groups, TRPV1 expression was weaker in the groups that received AMG517, but was still stronger than that in the uninjured side (**Figure 7**).

TRPV1 fluorescence intensity was stronger on the injured side than on the uninjured side at both time points measured following sciatic nerve crush injury, especially at 1 week ( $P < 0.001$ ). Injection with AMG517 before injury diminished TRPV1 expression, although it remained higher on

the injured side than the uninjured side ( $P < 0.05$ ; **Table 1**).

#### TRPV1 expression in the DRG after sciatic nerve crush injury

Immunofluorescence staining for TRPV1 in the DRG at L<sub>4</sub> showed that in contralateral DRGs, TRPV1-positive cells were mainly small neurons, accounting for  $10.00 \pm 1.59\%$  of DRG cells on the cross-section.

On the ipsilateral side, 1 and 2 weeks after injury, the number of TRPV1-positive cells in L<sub>4</sub> DRGs was noticeably greater than on the contralateral side. Moreover, TRPV1-positive cells were large, medium-sized and small (**Figure 8**). Following AMG517 pretreatment, there were fewer TRPV1-positive cells on the injured side than the uninjured side ( $P < 0.05$ ). No significant difference was observed in the percentage of TRPV1-positive neurons between the AMG 517-pretreated and vehicle-pretreated groups ( $P > 0.05$ ; **Table 2**).

#### Discussion

TRPV1 is considered to be one of the main targets in the treatment of pain (Szallasi and Sheta, 2012; Zakir et al., 2012; Martínez-Rojas et al., 2014; Sousa-Valente et al., 2014; Zielińska et al., 2015). Activated by a variety of noxious stimuli, TRPV1 is widely distributed in peripheral sensory nerve endings (Tsukagoshi et al., 2006; Axelsson et al., 2009; Neelands et al., 2010). Pain commonly occurs after peripheral nerve injury, and various intracellular signaling mechanisms are altered upon activation of TRPV1 (Li et al., 2004, 2015; Lee et al., 2012; Gu et al., 2013; Dussor et al., 2014; de Jong et al., 2014; Evangelista, 2014). We hypothesized that TRPV1 participates in nerve regeneration following peripheral nerve injury. To explore this relationship, we have investigated the expression of TRPV1 after nerve crush, and the effect of its antagonist AMG517 on nerve regeneration. Our results demonstrate that sciatic nerve crush injury enhances TRPV1 expression at the injury site and in the DRG, and that AMG517 pretreatment prevents the overexpression of TRPV1 and promotes axon regeneration after injury.

TRPV1 activation-induced changes in intracellular signaling pathways, cellular function and metabolism are not only limited to analgesia or interference in pain transmission, but are part of a multi-faceted, multi-channel, intracellular process. TRPV1 activation also produces anti-tumor effects, inducing cell cycle arrest, triggering cancer cell apoptosis, and inhibiting metabolism *via* intracellular signaling (Chen et al., 2012; Stock et al., 2012; de Jong et al., 2014; Skrzypski et al., 2014; Wu et al., 2014). Deactivation or inhibition of TRPV1 delays senescence and confers resistance to obesity (Suri and Szallasi, 2008; Lee et al., 2012; Baboota et al., 2014). In other words, this nociceptor is activated when damage occurs (Everaerts et al., 2011; Sadofsky et al., 2012; Iwashita et al., 2013; Ghazizadeh et al., 2014). In disease states, various harmful physical and chemical factors, generated at a local or systemic level, can activate TRPV1. Sciatic nerve crush injury is accompanied by disturbed blood flow, disintegration of tissue, accumulation of acidic metabolites and lipids, and an altered microenvironment. A wide variety of physical and chemical factors are involved, and various

myelin disintegration products are generated. These changes may cause activation or overactivation of TRPV1.

The results from the present study show that TRPV1 expression is markedly elevated locally after nerve injury, and may be accompanied by enhanced functional activity. However, whether the degree of neuropathic pain is associated with nerve regeneration after injury, and how enhanced TRPV1 expression affects nerve regeneration after injury, remains to be explored.

The analgesic baclofen, a GABAB agonist, promotes nerve regeneration in rats after sciatic nerve ligation (Sawynok and Dickson, 1983; Reis et al., 2006; Magnachi et al., 2014), suggesting that pain reduction is conducive to regeneration after injury. It was recently shown that GABA suppresses pathological TRPV1 activation and that genetic knockout or pharmacological inhibition of TRPV1 stimulates osteoblast activation and bone regeneration (Hanack et al., 2015). Together, this evidence suggests that severe pain produced by nerve injury may interfere with subsequent regeneration. In the present study, the increase in TRPV1 expression may constitute overexpression in local nerves and DRGs following sciatic nerve crush injury. TRPV1 overexpression is not only associated with severe pain, but may also impair regeneration. The potent and selective TRPV1 antagonist AMG517 promotes the regeneration of neurites and reduces TRPV1 expression. Our results further indicate that early nerve injury increased the overactivation of TRPV1, not only ameliorating pain, but also interfering nerve regeneration after peripheral damage.

AMG517 blocks calcium influx induced by TRPV1 activation, and in turn blocks intracellular TRPV1 signal transduction (Kort and Kym, 2012; Nash et al., 2012). One hour after AMG517 injection in the ipsilateral paw, the number of unmyelinated axons was notably elevated at the proximal and central injury sites, and typical C fiber clusters were present. Two weeks after injury, these features were more pronounced. Moreover, although AMG517 intervention reduced TRPV1 expression both locally and at the DRG, it did not restore it to pre-injury levels; this is consistent with the results from a previous study (Mitchell et al., 2010). These data suggest that changing the functional status of TRPV1 is more important for promoting nerve regeneration than decreasing TRPV1 overexpression after nerve injury.

TRPV1 is affected by agonists, synergists and antagonists, and has dual dose- and time-dependent effects. For example, a low dose or short duration of capsaicin stimulation can enhance TRPV1 expression and produce pain, whereas high-dose and long-duration capsaicin can diminish TRPV1 expression and relieve pain (Kaszas, et al., 2012; De Petrocellis and Moriello, 2013).

The change in number of axons shows that AMG517 contributes to nerve regeneration after sciatic nerve injury. Overexpression or overactivation of TRPV1 may interfere with axon regeneration after injury. Future studies should investigate the inhibitory effect on TRPV1 function of a single dose of a TRPV1 antagonist, and whether a threshold exists between physiological expression/activation and overexpression of TRPV1. Furthermore, many other factors are involved in

nerve regeneration; whether regeneration-related intracellular signaling pathways (such as NTF3, Erk1/2 and galanin) are altered by inhibition of TRPV1 remains to be investigated.

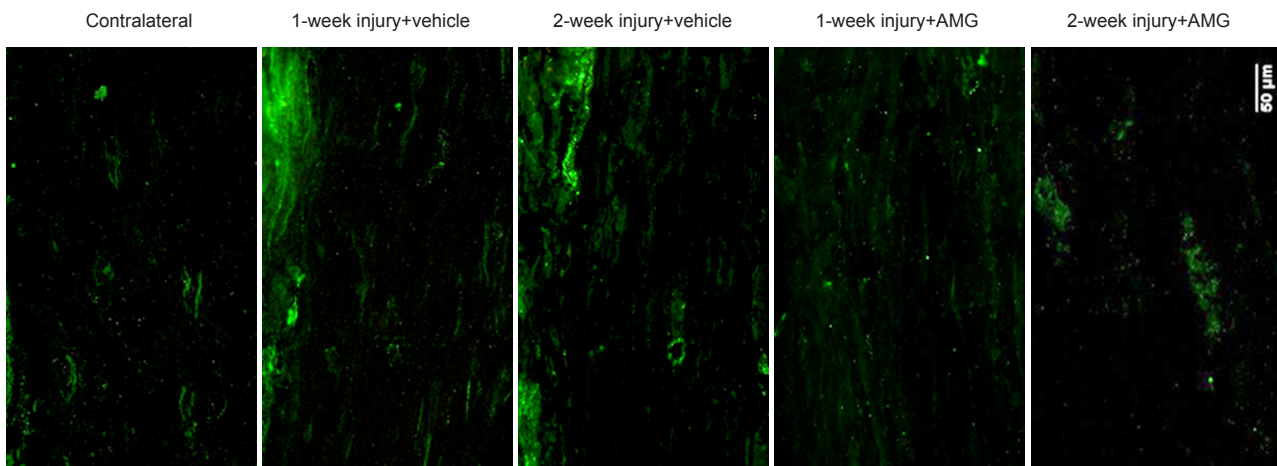
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**Author contributions:** *XQL obtained the funding, designed the study, provided technical support and revised the paper. FR participated in the immunofluorescence staining and paper writing. FR and HZ contributed equally to the animal surgery, sample processing and semithin-section preparation. CQ, MLG and HW participated in cryostat section and data analysis. All authors approved the final version of the paper.*

**Conflicts of interest:** *None declared.*

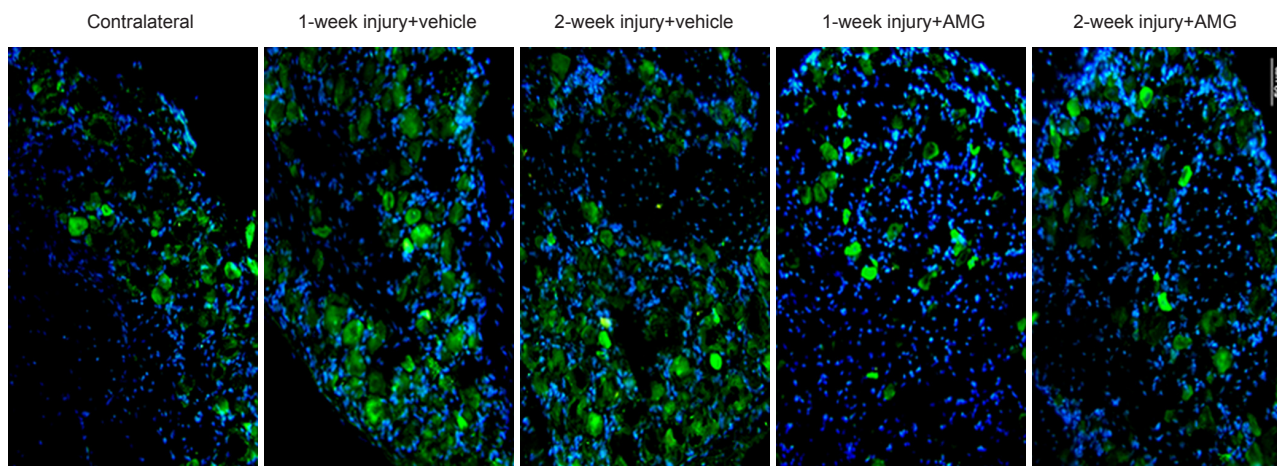
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**Figure 7 TRPV1 immunofluorescence in longitudinal sections of sciatic nerve with or without crush injury.**

No TRPV1 immunofluorescence was observed in the uninjured sciatic nerve, except for a weak positive reaction in the nerve trunk. At 1 and 2 weeks after injury, TRPV1 immunoreactivity (green fluorescence) was notably greater than in the uninjured nerve, especially near the epineurium. In AMG517-pretreated animals, TRPV1 immunoreactivity was weaker 1 and 2 weeks after injury than in the untreated groups, but still stronger than that in the uninjured side. TRPV1: Transient receptor potential cation channel subfamily V member 1; AMG: AMG517 (TRPV1 antagonist). Scale bar: 50 µm.



**Figure 8 Immunofluorescence staining for TRPV1 in the L<sub>4</sub> dorsal root ganglion following sciatic nerve crush injury.**

Some small TRPV1-positive neurons were visible in the uninjured nerve. At 1 and 2 weeks after injury + vehicle, the number of TRPV1-positive neurons in L<sub>4</sub> dorsal root ganglion (green fluorescence) was greater in the injured side than the control side. Moreover, some large-diameter neurons showed positive immunofluorescence. Fewer TRPV1-positive neurons were observed in AMG517 (TRPV1 antagonist)-pretreated groups than in the non-pretreated injury groups. Blue fluorescence (4',6'-diamidino-2-phenylindole, DAPI), nuclei. TRPV1: Transient receptor potential cation channel subfamily V member 1; AMG: AMG517. Scale bar: 50 µm.

**Table 1 AMG517 (TRPV1 antagonist) pretreatment resulted in weaker TRPV1 immunoreactivity (gray value) in injured sciatic nerve than in non-pretreated injured nerve**

Group	Ipsilateral (right)	Contralateral (left)
1-week injury+vehicle	0.034±0.001**	0.022±0.002
2-week injury+vehicle	0.030±0.002*	0.019±0.003
1-week injury+AMG517	0.028±0.002*	0.020±0.001
2-week injury+AMG517	0.026±0.004*	0.018±0.001

\* $P < 0.05$ , \*\* $P < 0.01$ , vs. contralateral (mean ± SEM,  $n = 10$ ; one-way analysis of variance and the least significant difference test).

**Table 2 Effect of AMG517 (TRPV1 antagonist) pretreatment on percentage (%) of TRPV1-positive neurons in L<sub>4</sub> dorsal root ganglion**

Group	Ipsilateral (right)	Contralateral (left)
1-week injury+vehicle	10.63±1.59	21.79±2.08**
2-week injury+vehicle	10.32±1.51	18.73±0.24*
1-week injury+AMG517	13.36±1.62	17.36±1.87*
2-week injury+AMG517	9.38±1.31	16.73±1.85*

\* $P < 0.05$ , \*\* $P < 0.01$ , vs. ipsilateral (mean ± SD,  $n = 10$ ; one-way analysis of variance and the least significant difference test). TRPV1: Transient receptor potential cation channel subfamily V member 1; AMG: AMG517.

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