Motor neuron-specific RhoA knockout delays degeneration and promotes regeneration of dendrites in spinal ventral horn after brachial plexus injury

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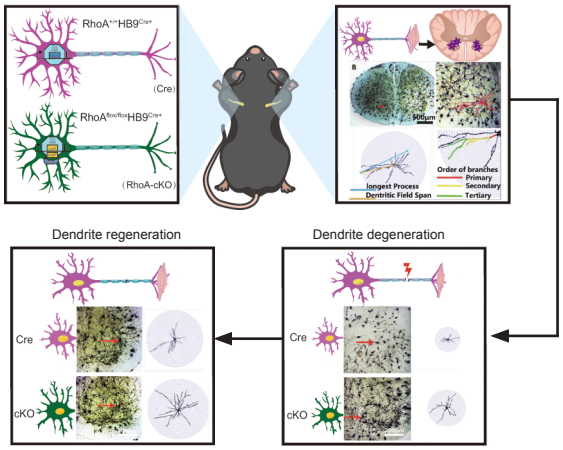
From the Contents

Graphical Abstract

RhoA knockout in motor neurons attenuates dendrite degeneration and promotes dendrite regeneration after peripheral nerve injury



Abstract

Dendrites play irreplaceable roles in the nerve conduction pathway and are vulnerable to various insults. Peripheral axotomy of motor neurons results in the retraction of dendritic arbors, and the dendritic arbor can be re-expanded when reinnervation is allowed. RhoA is a target that regulates the cytoskeleton and promotes neuronal survival and axon regeneration. However, the role of RhoA in dendrite degeneration and regeneration is unknown. In this study, we explored the potential role of RhoA in dendrites. A line of motor neuronal RhoA conditional knockout mice was developed by crossbreeding *HB9*$^{Cre+}$ mice with *RhoA*$^{flox/flox}$ mice. We established two models for assaying dendrite degeneration and regeneration, in which the brachial plexus was transection or crush injured, respectively. We found that at 28 days after brachial plexus transection, the density, complexity, and structural integrity of dendrites in the ventral horn of the spinal cord of RhoA conditional knockout mice were slightly decreased compared with that in Cre mice. Dendrites underwent degeneration at 7 and 14 days after brachial plexus transection and recovered at 28–56 days. The density, complexity, and structural integrity of dendrites in the ventral horn of the spinal cord of RhoA conditional knockout mice recovered compared with results in Cre mice. These findings suggest that RhoA knockout in motor neurons attenuates dendrite degeneration and promotes dendrite regeneration after peripheral nerve injury.

Key Words: brachial plexus; conditional knockout; degeneration; dendrites; motor neuron; peripheral nerve injury; regeneration; RhoA; spinal cord; ventral horn

Introduction

Peripheral nerve injury (PNI) is a common clinical traumatic disease that results in motor and sensory function defects and life-long disabilities. While the peripheral nervous system has a certain capacity to regenerate and re-innervate targets, the functional repair of PNI in patients in the clinic is still unsatisfactory (Lopes et al., 2022). A meta-analysis of 23 clinical studies demonstrated that only 51.6% of median or ulnar nerve-injured patients achieved successful motor recovery (Ruijs et al., 2005). The somatic motor nerve conduction pathway includes neurons in the motor cortex whose axons transmit signals to the dendrites of spinal motor neurons; the spinal motor neurons extend their axons to innervate the target muscles. Previous studies have reported that the dendrites of motor neurons exhibit disintegration or retraction after PNI, and the dendrite regeneration can be evoked after the axonal regeneration was achieved to re-innervate the target muscles (Zhang et al., 2023). Therefore, dendritic plasticity plays a critical role in neural circuit reconstruction after PNI. However, the molecular mechanisms regulating dendrite plasticity after PNI have not been thoroughly explored.

RhoA is an important molecular switch that regulates cytoskeleton dynamics and exhibits various functions in different cells of the nervous system (Xu et al., 2021). Studies have shown that RhoA plays a key role in axonal development and regeneration and in dendrite development (Wang et al., 2022b). However, whether RhoA is involved in dendrite degeneration and regeneration has not been investigated.

While previous studies have explored the mechanisms of axonal regeneration and dendrite development, some findings have indicated that the molecules involved in axon and dendrite regeneration might be different (Brar et al., 2022; Wang et al., 2022a). For example, one study reported that dendrite regeneration does not require the injury-sensing c-Jun N-terminal kinase signaling pathway, which is essential for axon regeneration (Stone et al., 2014). Moreover, the molecules controlling dendrite regeneration might be different from those of dendrite development because of alterations in the micro-environment and the intrinsic cellular patterning (Thompson-Peer et al., 2016).

Considering that the RhoA pathway is well known to play important roles in axonal regeneration, we aimed to determine whether RhoA is also involved in

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dendrite regeneration or if RhoA, similar to the above-mentioned molecules, is not involved in this process. To determine the potential role of RhoA in dendrites, we developed a transgenic mouse line in which RhoA was knocked out only in motor neurons and established two different PNI mouse models for assaying dendrite degeneration and regeneration, in which the brachial plexus was transection or crush injured, respectively. We investigated the morphologic changes of dendritic arbors in the spinal ventral horn after injury.

Methods

Animals

C57BL/6J mice (males and females, 8 weeks old, weight 20–30 g) were provided by the Animal Center of Southern Medical University (license No. SCXK (Yue) 2016-0041). *RhoA*$^{flox/+}$ mice were constructed by Cyagen Biosciences Company (Suzhou, China) and *HB9*$^{Cre+}$ mice were from Jackson Laboratory (Bar Harbor, ME, USA, Stock No. 006600, RRID: IMSR_JAX:006600). All animal procedures were carried out with the approval of the Southern Medical University Animal Care and Use Committee (approval No. SMU-L2018158) in September 2018. in accordance with the Animal Research Reporting of *In Vivo* Experiments (ARRIVE) guidelines for the ethical treatment of the animals (Percie du Sert et al., 2020). The mice were housed under specific pathogen-free conditions at 22 ± 1°C and 55% relative humidity on a 12-hour light/dark cycle. Mice were housed in standard cages (maximum of four males per cage and maximum of five females per cage) and provided with food and water *ad libitum*.

Motor neuron-specific conditional knockout (cKO) mice were generated by crossing *RhoA*$^{flox/+}$ mice and *HB9*$^{Cre+/-}$ mice. The F2 generation mice with genotyping of *RhoA*$^{flox/flox}$; *HB9*$^{Cre+}$ (cKO) or *RhoA*$^{+/+}$;*HB9*^{Cre} (Cre) were used in experiments.

Surgical procedures for brachial plexus injury We established two different PNI mouse models for assaying dendrite degeneration and regeneration, respectively. One model was established by brachial plexus transection, which is a simplified PNI model with no potential for nerve regeneration that may interrupt the analysis of dendrite degeneration. The second model is the brachial plexus crush model, in which the dendrite regeneration is in the chronic stage after injury. The surgical procedures were performed as previously described, with slight modifications (Zhong et al., 2022). Briefly, the mice were intraperitoneally injected with tribromoethanol (180 mg/kg body weight, Sigma-Aldrich, St. Louis, MO, USA). Mice were held in the supine position; the right side of the brachial plexus was exposed, and the C5–T1 spinal nerve roots were separated under a surgical microscope. The trunks of the brachial plexus were cut with a pair of micro-scissors (for the transection model) or crushed with hemostatic forceps for 1 minute (for the crush model). The nerve injury site was determined at 3 mm from the intervertebral foramen. During surgery, a heat pad was used to prevent temperature loss. For anti-inflammatory and analgesic treatment, the mice were administrated with aspirin (120 mg/kg body weight, SATO, Tokyo, Japan) for 5 days after surgery.

At 28 days post-injury (dpi) after brachial plexus transection or 7, 14, 28, and 56 dpi after brachial plexus crush, the mice were euthanized. The C6–C7 spinal cord was collected for immunofluorescence staining or Golgi-Cox staining.

Immunofluorescence staining At indicated time points after injury, mice (n = 5 per group) were euthanized with an overdose injection of tribromoethanol (250 mg/kg body) and transcardially perfused with 4% paraformaldehyde. The C6–C7 segments of the spinal cord were harvested and cryosectioned for conventional immunofluorescence staining as described previously (Liu et al., 2022b). Briefly, the sections were incubated with primary antibody against rabbit anti-microtubule associated protein 2 (MAP2; 1:400, Sigma, St. Louis, MO, USA, Cat# 05-346, RRID: AB_309685) overnight at 4°C followed by incubation with goat anti-rabbit Alexa-conjugated 568 fluorescence secondary antibody (1:400; Molecular Probes, Eugene, OR, USA, Cat#A-11011, RRID: AB_143157) for 2 hours at room temperature. In some assays, double immunofluorescence staining was performed with mouse anti-RhoA (1:100; Cat# sc-418, Santa Cruz Biotechnology, Dallas, TX, USA, RRID:AB_628218) and rabbit anti-NeuN (1:400; Abcam, Cambridge, UK, Cat# ab104224, RRID: AB_10711040), followed by staining with goat anti-mouse Alexa-conjugated 568 (1:400; Invitrogen, Carlsbad, CA, USA, Cat# A-11004, RRID: AB_2534072) and goat anti-rabbit Alexa-conjugated 488 (1:400; Invitrogen, Cat#A-11008, RRID: AB_143165) fluorescence secondary antibodies.

Golgi-Cox staining

Mice (n = 5 for each group) were euthanized with tribromoethanol; paraformaldehyde perfusion was not performed. The C6–C7 segments of the spinal cord were quickly dissected and Golgi-Cox staining was performed following the instructions of Hito Golgi-Cox OptimStain™ Kit (HitoBiotec, Kingsport, TN, USA) (Sun et al., 2021). Briefly, the tissue was immersed in solution #1/2 (1:1 mixture) at 4°C for 5 days, immersed in isopentane, and quickly frozen for 30 seconds in a liquid nitrogen tank. The tissue was cut into 90 µm sections with a cryostat (Leica, Wetzlar, Germany) and mounted onto gelatin-coated slices. After rinsing with solution #3–5 successively, dehydration with ethanol, and incubation in xylene, the slices were mounted with neutral gum. All operations were performed in dark conditions.

Morphometric analysis of dendritic structure

Under a fluorescent microscope (Leica, Wetzlar, Germany), a 200 µm × 200 µm digital image was captured in the spinal anterolateral horn of each MAP2 immunostained section, as shown in **Figure 1A**. Using Photoshop 8.0 software (Adobe, San Jose, CA, USA), three vertical lines and three horizontal lines at intervals of 50 µm were superimposed onto the images. The number of MAP2-positive dendrites that intercepted with the superimposed lines was counted and indicated the density of dendrites. To determine dendritic integrity, the number of dendrites with more than two intersections was counted.

After Golgi-Cox staining, the neurons were traced and analyzed by the Sholl technique as described previously (Han et al., 2019). Images (730 µm × 730 µm) of each section were captured from the anterolateral horn of spinal cord under a light microscope (Leica). Five sections from each sample (the C6–C7 spinal cord) and five mice from each group were used for evaluation. Two neurons with structural integrity in each section were selected; the cell body and dendrites were traced and the redundant background in the images was removed using Photoshop software. The images were subjected to Sholl analysis using ImageJ software (v8.1, National Institutes of Health, Bethesda, MD, USA) (Schneider et al., 2012). The length of the longest dendrite, total length of all dendrites, dendritic span (the longest diameter of the range covered by all dendrites), and the number of dendritic primary, secondary, and tertiary branches were quantified to indicate the dendrite arborization complexity and structural integrity of each neuron (**Figure 1B**; Srinivasan et al., 2020).

Statistical analysis

All statistical analyses were performed with GraphPad Prism software (v8.0.1, GraphPad Software, San Diego, CA, USA, www.graphpad.com) and SPSS (v26.0.0.0, IBM, Armonk, NY, USA) using one-way analysis of variance followed by Bonferroni's multiple test. A P value of < 0.05 was considered statistically significant.

Results

Confirmation of RhoA knockout in spinal motor neurons

To investigate the role of RhoA in dendrite degeneration and regeneration of spinal motor neurons after PNI, we specifically knocked out RhoA in motor neurons by generating *RhoA*$^{flox/flox}$;*HB9*$^{Cre+}$ (cKO) mice, as described in Methods. Our preliminary data indicated RhoA is expressed at low levels in naïve adult motor neurons but is up-regulated after PNI. As shown in **Figure 1C**, in the Cre control mice, RhoA was highly expressed in the spinal ventral horn at 7 dpi. In contrast, neurons in the spinal ventral horn of the cKO mice were negative for RhoA, while the surrounding cells were positive for RhoA. These data demonstrated that RhoA had been specifically knocked out in motor neurons in the cKO mice.

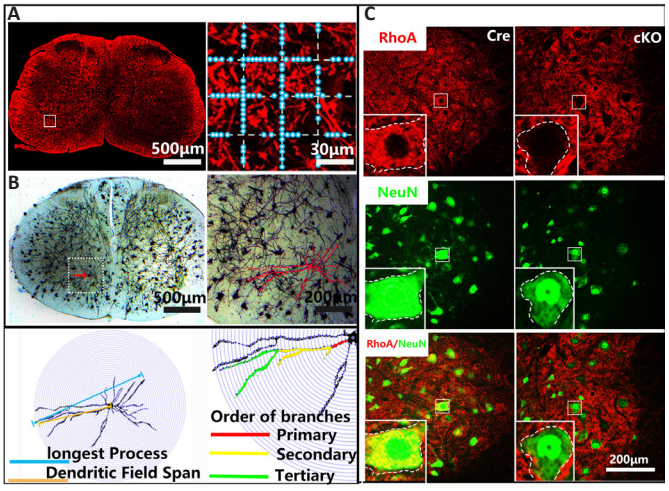

Figure 1 | Quantitative methods on the MAP2 immunostaining and Golgi-Cox staining images and confirmation of RhoA conditional knockout in spinal motoneurons.

(A) The method for quantifying dendritic density in MAP2 immunofluorescence images. The blue dots indicate each point where the dendrites cross the grid and were counted. Scale bars: 500 µm, 30 µm (enlarged image). (B) Quantification of dendritic arbor in Golgi-Cox images. Scale bars: 500 µm, 200 µm (enlarged image). (C) Immunofluorescence for RhoA (red) and NeuN (green) in the spinal ventral horn of Cre and cKO mice (n = 3 for each group). The dotted line represents the edge of the neurons. Scale bar: 200 µm. cKO: Conditional knockout; MAP2: microtubule-associated protein 2.

RhoA knockout attenuates spinal motor neuronal dendrite loss and fragmentation after brachial plexus transection

To evaluate the role of RhoA in motor neuron dendrite degeneration after PNI, we first examined the overall profile of dendrite distribution in the spinal cord by MAP2 immunostaining. After brachial plexus transection, the density of MAP2-positive dendrites was markedly decreased in the spinal ventral horn of the injured side compared with the uninjured side in both Cre and cKO groups; many dendrites were broken into short segments (**Figure 2A**). Quantification of the dendrite density revealed that dendrite density (quantified by dendrite intersections on MAP immunostaining, as shown in Methods) and fragmentation were higher in the cKO mice compared with the Cre mice (**Figure 2B**).

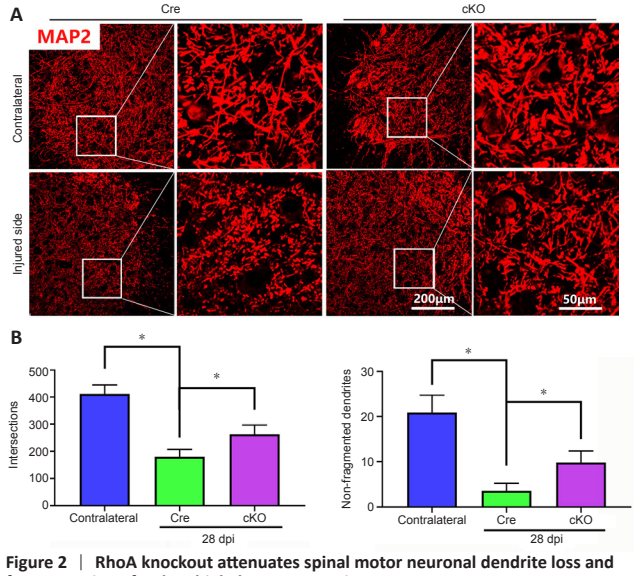

Figure 2 | RhoA knockout attenuates spinal motor neuronal dendrite loss and fragmentation after brachial plexus transection.
(A) Representative images of MAP2 immunofluorescence (red) showing the dendrites in the uninjured and injured sides in Cre and cKO groups after brachial plexus transection. Scale bars: 200 μm, 50 μm (enlarged images). (B) Quantification of the intersections of MAP2-positive dendrites and the superimposed lines (non-fragmented dendrites) and the number of dendrites with more than two intersections in Figure 1A. Data are expressed as the mean ± SEM (n = 5 for each group). *P < 0.05 (one-way analysis of variance followed by Bonferroni's multiple test). cKO: Conditional knockout; MAP2: microtubule-associated protein 2.

RhoA knockout alleviates the degeneration of dendrite arborization after brachial plexus transection

Similar to the MAP2 immunostaining results, the Golgi staining images also showed that dendrites in the injury side of the cKO group were denser than that of the Cre group (**Figure 3**). The individual neurons with intact soma were tracked out with their continuous dendrites and then subjected to Sholl analysis. The results showed that the intersections of dendrites with concentric circles at each radius were dramatically decreased on the injured side compared with the uninjured side in both the cKO group and Cre group (**Figure 3**).

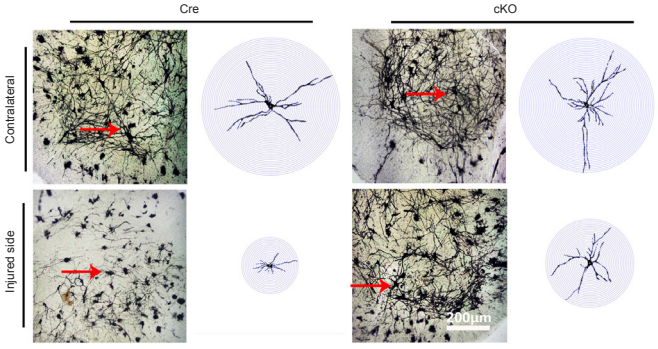

Figure 3 | RhoA knockout alleviates the degeneration of dendrite arborization after brachial plexus transection.
Representative images of Golgi-Cox staining and the traced single neurons with dendrites in uninjured and injured sides of Cre and cKO groups 28 days post-brachial plexus transection injury are shown. The loss of dendritic structure of spinal motoneurons in cKO mice was ameliorated compared with results in Cre mice. The red arrows indicate the neurons that are traced in the images on the right. Scale bar: 200 μm. cKO: Conditional knockout.

Further analysis revealed that while there were no differences in the intersections on the uninjured side between the cKO group and Cre group, the number of intersections were different between the two groups on the injured side (**Figure 4A**). The dendritic arbors in the cKO group were longer and had more intersections at each radius compared with those in the Cre group, as determined by Sholl analysis. Moreover, the length of the longest process, total length of all dendrites, dendritic field span, and the numbers of primary branches, secondary branches, and tertiary branches were all dramatically higher in the injured side of cKO group compared with those of Cre group (**Figure 4B**). These results indicate that spinal motor neuronal dendrite arborization degenerated dramatically after brachial plexus transection, but the degeneration was alleviated in the cKO group.

RhoA knockout enhances restoration of dendrite density and integrity after brachial plexus crush injury

To investigate the role of RhoA in dendrite reconstruction after PNI, we used the brachial plexus crush model. MAP2 staining results showed that the

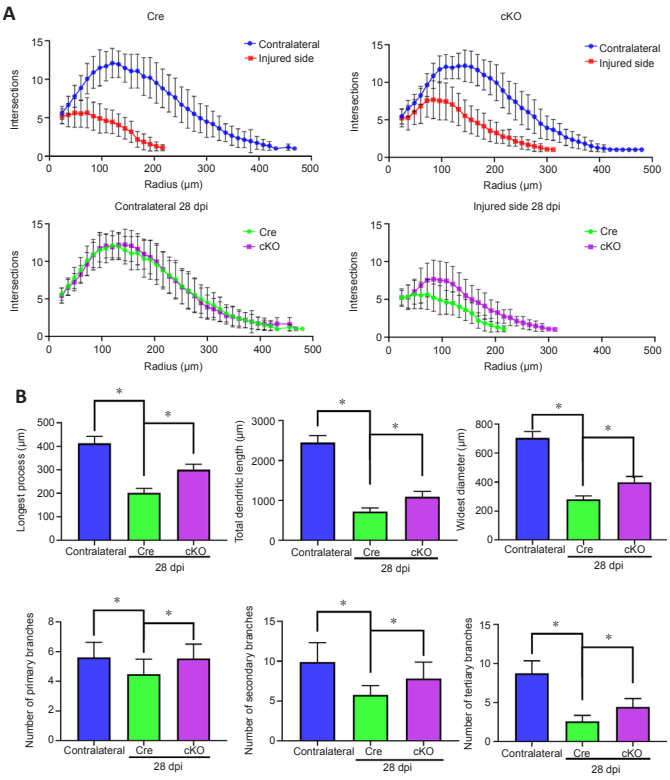

Figure 4 | Degeneration of spinal motor neuronal dendrite arborization after brachial plexus transection is alleviated in mice with RhoA knockout.
Sholl analysis was performed on the dendrites of traced single neurons in Cre and cKO groups at 28 days post-brachial plexus transection injury. (A) The Sholl curves showed the differences between uninjured and injured sides of the Cre and cKO groups. (B) Quantification of the length of the longest dendrite, total length of all dendrites, dendritic span, and the number of dendritic first, secondary and tertiary branches. Data are expressed as the mean ± SEM (n = 5 for each group). *P < 0.05 (one-way analysis of variance followed by Bonferroni's multiple test). cKO: Conditional knockout; dpi: days post-injury.

dendrite density and integrity of the Cre mice decreased from 7 dpi to 14 dpi; the density increased from 28 dpi to 56 dpi while the integrity was maintained at a low level (**Figure 5A**). Quantification showed that the decreases in dendrite density and integrity at 7 and 14 dpi were attenuated in the cKO mice compared with those of Cre group; dendrite density and integrity were also increased at 28 and 56 dpi in the cKO group (**Figure 5B**).

RhoA knockout accelerates the recovery of dendrite arborization in the brachial plexus crush injury model

To further investigate the role of RhoA in dendrite regeneration, Golgi-Cox staining and Sholl analysis were performed on the spinal cord sections of brachial plexus crush model. As expected, dendrite arborization of the uninjured side was similar at all time points in both cKO and Cre groups. On the injured side, dendrite arborization retracted from 7 dpi to 14 dpi and then showed recovery from 28 dpi to 56 dpi, and the recovery of dendrite arborization in the cKO group improved to a higher degree than the Cre group (**Figure 6**). These results were consistent with the pattern of dendrite density and integrity shown by MAP2 staining.

We performed a quantitative analysis to examine the difference in dendritic complexity and integrity between the two groups by Sholl analysis. The results showed that, except for the number of primary branches of the two groups that did not show obvious change after brachial plexus crush injury, other parameters (including length of the longest dendrite, total dendrite length, dendrite field span, numbers of secondary and tertiary branches) initially decreased and then increased from 7 dpi to 56 dpi. Notably, all parameters were higher in the cKO group compared with the Cre group at all time points (**Figure 7**).

Together, these data indicate that RhoA plays an important role in the fate of spinal motor neuron dendrites after PNI, and RhoA deletion alleviates dendrite degeneration and enhances dendrite regeneration.

Discussion

Neuronal dendrites are vulnerable to various physical or chemical insults during degeneration, stroke, and trauma (Beckers and Moons, 2019; Liu et al., 2022a). While PNI does not directly harm motor neuronal dendrites, previous studies have demonstrated that peripheral axotomy of motor neurons results in retraction of dendritic arbors, and the dendritic arbors can be re-expanded when reinnervation is allowed (Brännström et al., 1992). Research on the dendrite retraction and regeneration is limited, with studies only reporting

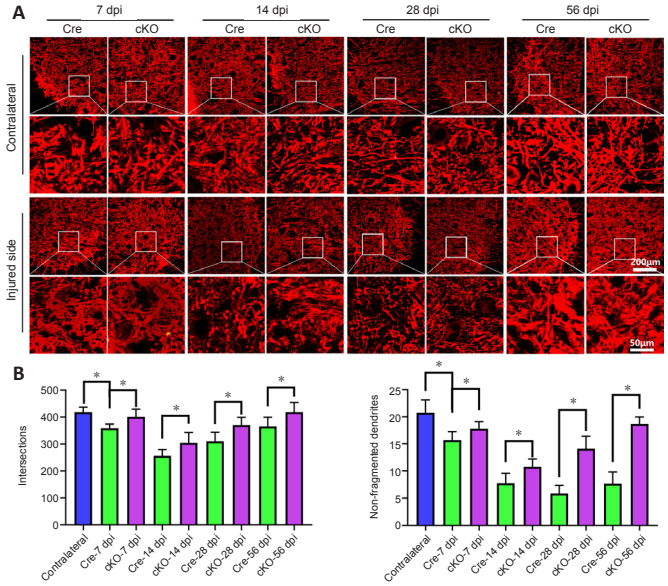

Figure 5 | **RhoA knockout enhances restoration of dendrite density and integrity after brachial plexus crush injury.**
(A) MAP2 immunostaining reveals dendrite density and fragmentation in the spinal ventral horn 7, 14, 28, and 56 days post-brachial plexus crush injury in the uninjured and injured sides of both Cre and cKO groups post injury. Scale bars: 200 μm, 50 μm (enlarged images). (B) Quantification of the intersections of MAP2-positive dendrites with the superimposed lines and the numbers of the dendrites with more than two intersections. Data are expressed as the mean ± SEM (n = 5 for each group). *P < 0.05 (one-way analysis of variance followed by Bonferroni's multiple test). cKO: Conditional knockout; dpi: days post-injury; MAP2: microtubule-associated protein 2.

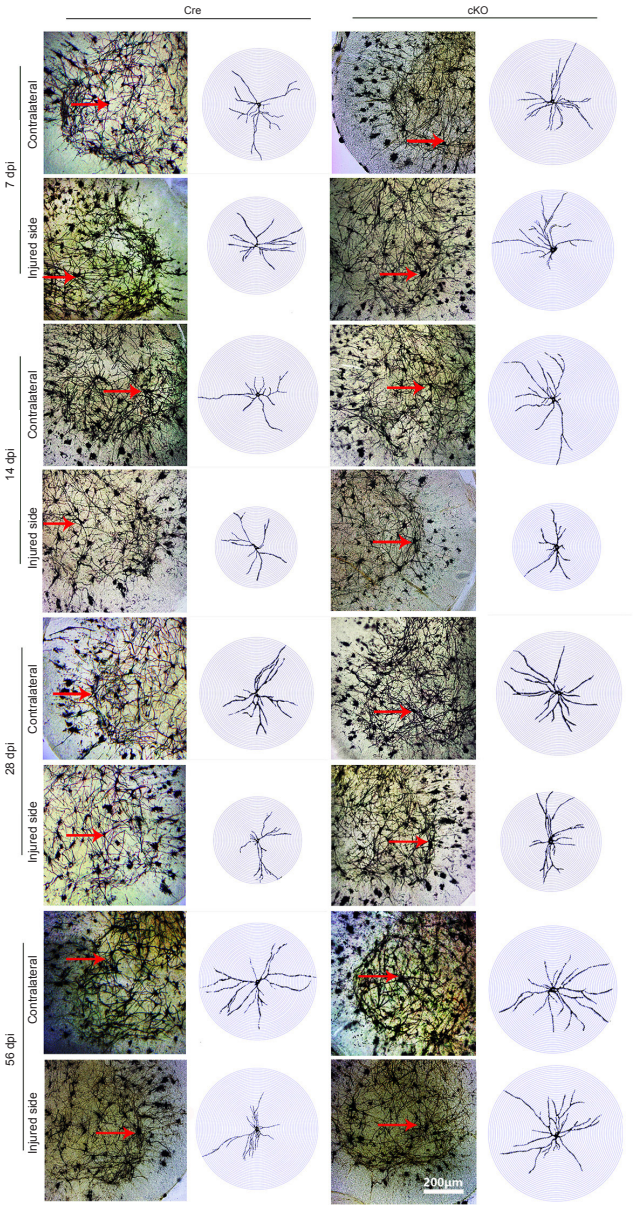

Figure 6 | **RhoA knockout accelerates the recovery of dendrite arborization in the model of brachial plexus crush injury.**
Representative images of Golgi-Cox staining and the traced single neurons with dendrites of uninjured and injured sides of Cre and cKO groups at 7, 14, 28 and 56 days post-brachial plexus crush injury. Compared with Cre mice, the length and structural branches of dendrites were repaired more efficiently in cKO mice. The red arrows indicate the neurons traced in the right images. Scale bars: 200 μm. cKO: Conditional knockout; dpi: days post-injury.

the dendritic stem diameter, length, or volume decrease after nerve injury (Furusawa and Emoto, 2020; Zhao et al., 2021). The detailed changes in dendritic integrity and complexity of the axotomized motor neuron, and the molecular mechanisms involved in regulating dendritic degeneration and regeneration after PNI, are still largely unexplored.

We recently reported the correlation of time course and distance of brachial plexus transection on spinal motor neuronal dendritic degeneration, which includes dendritic density and integrity, in the spinal ventral horn and the length, number, and complexity of motor neuron dendrites (Li et al., 2022). We found that dendrite degeneration occurs and gets worse in spinal motor neurons after PNI over time. Furthermore, the degree of dendrite degeneration reduced with the extension of the distance between injury site and spinal cord. Therefore, compared with previously reported models of medial gastrocnemius nerve transection, the brachial plexus injury model might be more suitable for the study of the molecular mechanism in dendrite degeneration and regeneration after PNI, as the lesion site can be achieved closer to the spinal cord (Brännström et al., 1992) or sciatic nerve transection (Huang et al., 1997). Moreover, traumatic brachial plexus injury is a common nerve injury in clinic (Zhang et al., 2022). Therefore, we choose this model for the present study.

Increasing studies have shown that the mechanism underlying dendrite regeneration is independent of molecular mechanisms involved in axon regrowth. For example, one study reported that some molecules controlling axonal regrowth do not affect dendrite regeneration (Stone et al., 2014). The authors developed methods to remove all dendrites from da neurons, a type of soma sensory neurons in *Drosophila*, and found that dual leucine zipper kinase, c-Jun N-terminal kinase, and c-fos are dispensable for dendrite regeneration, even though these factors are essential for axon regeneration (Hammarlund et al., 2009). Another study reported that spastin, atlastin, and endoplasmic reticulum relocalization are involved in axon but not dendrite regeneration (Rao et al., 2016). Most recently, Brar et al. (2022) severed both axon and dendrites of PVD neurons in *Caenorhabditis elegans* that are responsible for proprioception and harsh touch sensation and found that the mechanisms driving dendrite regeneration are independent of molecular mechanisms involving axon regrowth.

As the RhoA pathway plays important roles in axonal regeneration, in this study, we explored whether RhoA functions in dendrite regeneration or is dispensable for dendrite regeneration. We also explored the role of RhoA in dendrite degeneration, one of the main causes of functional defects after PNI (Xue et al., 2022). We first developed a line of RhoA cKO mice, with RhoA knockout in motor neurons, to determine the role of RhoA in motor neuronal dendrites. MAP2 immunohistochemistry, Golgi-Cox staining, and morphological quantitative analysis revealed that the brachial plexus transection-derived dendritic degeneration (decrease of dendrite density, arborization complexity, and structural integrity) in the spinal ventral horn was attenuated in RhoA cKO mice compared with Cre mice. In the brachial plexus crush model, the dendrites degenerated at 7 and 14 dpi but underwent regeneration from 28 dpi to 56 dpi. The dendritic regeneration, indicated by density, arborization complexity, and structural integrity, was enhanced in the RhoA cKO mice compared with Cre mice. This study has some limitations. Such as which downstream pathway(s) is/are involved in RhoA regulating the progress of dendritic degeneration and regeneration after nerve injury remains unknown. In conclusion, this study illustrated that RhoA knockout in motor neurons attenuates dendrite degeneration and promoted dendrite regeneration after PNI in a mouse model. These findings suggest that RhoA plays similar roles in dendrites and axons after PNI. RhoA inhibition may be a prospective strategy for nerve repair with potential effects on axonal regeneration and dendrite regeneration.

Author contributions: *Study conception and design: JG; animal surgery: ML, JL, YX; immunohistochemistry: ML, JX, YZ; Golgi-Cox staining: ML, JL, AO; data analysis: ML, JZ, LF, XM, XW, LZ; manuscript writing: ML, JG. All authors approved the final version of the manuscript.*
Conflicts of interest: *The authors declare no conflict of interest.*
Data availability statement: *All relevant data are within the paper.*

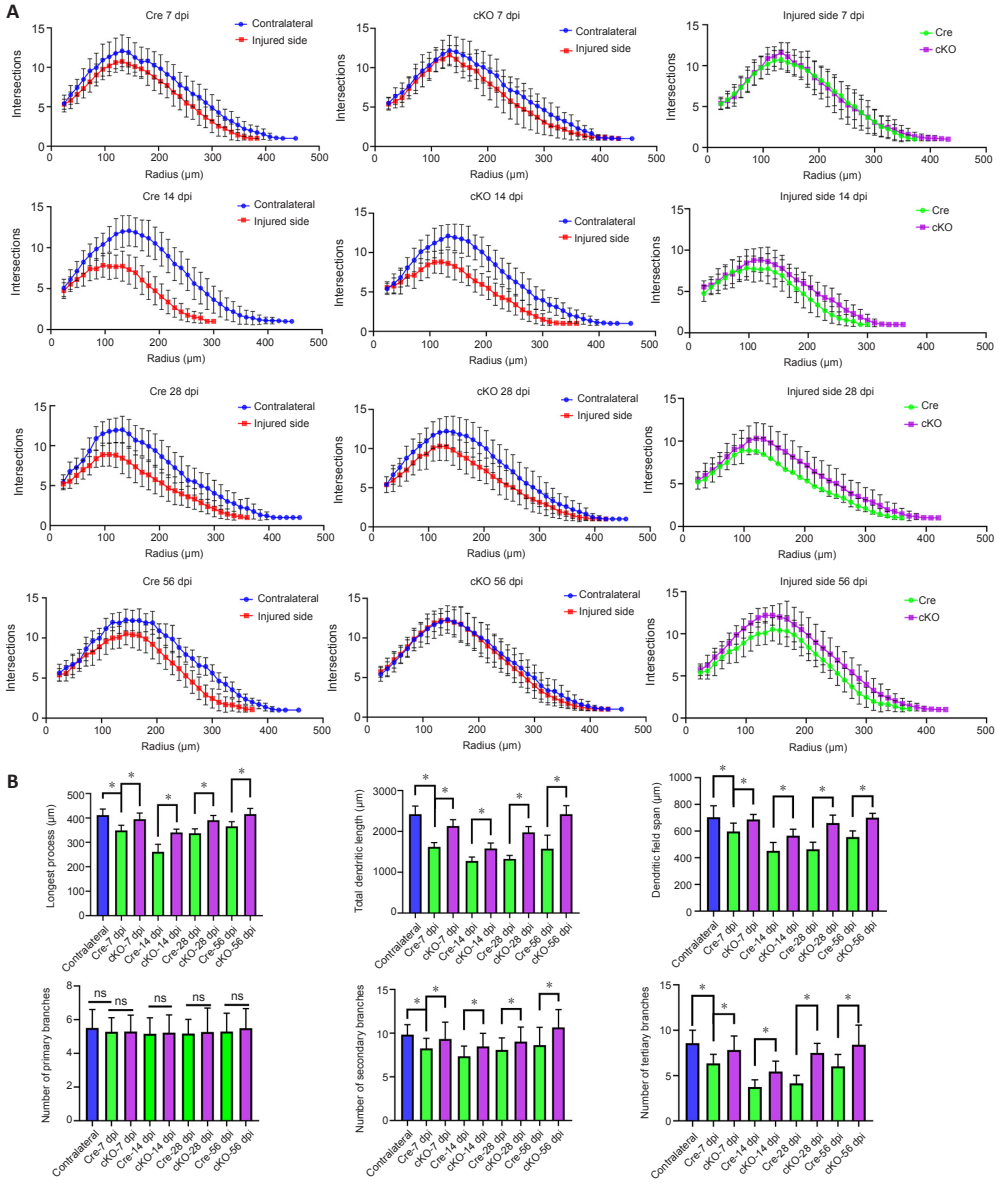

Figure 7 | Regeneration of spinal motor neuronal dendrite arborization after brachial plexus transection is enhanced in mice with RhoA knockout.

Sholl analysis of the dendrites of traced single neurons in Cre and cKO groups at 7, 14, 28, and 56 days post-brachial plexus crush injury. (A) The Sholl curves show differences between the uninjured and injured sides and differences in the injured side between Cre and cKO groups. (B) Quantification of the length of the longest dendrite, total length of all dendrites, dendritic span, and the number of dendritic primary, secondary and tertiary branches. Data are expressed as the mean ± SEM (n = 5 for each group). *P < 0.05 (one-way analysis of variance followed by Bonferroni's multiple test). cKO: Conditional knockout; dpi: days post-injury.

References

Beckers A, Moons L (2019) Dendritic shrinkage after injury: a cellular killer or a necessity for axonal regeneration? Neural Regen Res 14:1313-1316.

Brännström T, Havton L, Kellerth JO (1992) Changes in size and dendritic arborization patterns of adult cat spinal alpha-motoneurons following permanent axotomy. J Comp Neurol 318:439-451.

Brar HK, Dey S, Bhardwaj S, Pande D, Singh P, Dey S, Ghosh-Roy A (2022) Dendrite regeneration in C. elegans is controlled by the RAC GTPase CED-10 and the RhoGEF TIAM-1. PLoS Genet 18:e1010127.

Furusawa K, Emoto K (2020) Scrap and build for functional neural circuits: spatiotemporal regulation of dendrite degeneration and regeneration in neural development and disease. Front Cell Neurosci 14:613320.

Hammarlund M, Nix P, Hauth L, Jorgensen EM, Bastiani M (2009) Axon regeneration requires a conserved MAP kinase pathway. Science 323:802-806.

Han Q, Ordaz JD, Liu NK, Richardson Z, Wu W, Xia Y, Qu W, Wang Y, Dai H, Zhang YP, Shields CB, Smith GM, Xu XM (2019) Descending motor circuitry required for NT-3 mediated locomotor recovery after spinal cord injury in mice. Nat Commun 10:5815.

Huang Y, Wang S, Chu X (1997) Studies on the changes of morphometry and neuropeptide of spinal neurons after peripheral nerve injury. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 11:6-9.

Li M, Xu JW, Zou Y, Guo JS (2022) The effects of brachial plexus transection on the structure and morphology degeneration of spinal motoneuron dendrites in mice. Zhongguo Linchuang Jiepou Xue Zazhi 40:277-285.

Liu HH, Hsu CH, Jan LY, Jan YN (2022a) A photo-switchable assay system for dendrite degeneration and repair in Drosophila melanogaster. Proc Natl Acad Sci U S A 119:e2204577119.

Liu J, Li L, Zou Y, Fu L, Ma X, Zhang H, Xu Y, Xu J, Zhang J, Li M, Hu X, Li Z, Wang X, Sun H, Zheng H, Zhu L, Guo J (2022b) Role of microtubule dynamics in Wallerian degeneration and nerve regeneration after peripheral nerve injury. Neural Regen Res 17:673-681.

Lopes B, Sousa P, Alvites R, Branquinho M, Sousa AC, Mendonça C, Atayde LM, Luís AL, Varejão ASP, Maurício AC (2022) Peripheral nerve injury treatments and advances: one health perspective. Int J Mol Sci 23:918.

Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, et al. (2020) The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol 18:e3000410.

Rao K, Stone MC, Weiner AT, Gheres KW, Zhou C, Deitcher DL, Levitan ES, Rolls MM (2016) Spastin, atlastin, and ER relocalization are involved in axon but not dendrite regeneration. Mol Biol Cell 27:3245-3256.

Ruijs AC, Jaquet JB, Kalmijn S, Giele H, Hovius SE (2005) Median and ulnar nerve injuries: a meta-analysis of predictors of motor and sensory recovery after modern microsurgical nerve repair. Plast Reconstr Surg 116:484-494; discussion 495-496.

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. Nat Methods 9:671-675.

Srinivasan A, Srinivasan A, Ferland RJ (2020) AutoSholl allows for automation of Sholl analysis independent of user tracing. J Neurosci Methods 331:108529.

Stone MC, Albertson RM, Chen L, Rolls MM (2014) Dendrite injury triggers DLK-independent regeneration. Cell Rep 6:247-253.

Sun Z, Wang B, Chen C, Li C, Zhang Y (2021) 5-HT6R null mutatrion induces synaptic and cognitive defects. Aging Cell 20:e13369.

Thompson-Peer KL, DeVault L, Li T, Jan LY, Jan YN (2016) In vivo dendrite regeneration after injury is different from dendrite development. Genes Dev 30:1776-1789.

Wang K, Zhi XD, Wang W (2022a) Effect and mechanism of human amniotic epithelial cells on nerve injury repair. Zhongguo Zuzhi Gongcheng Yanjiu 26:4046-4051.

Wang Q, Song LJ, Ding ZB, Chai Z, Yu JZ, Xiao BG, Ma CG (2022) Advantages of Rho-associated kinases and their inhibitor fasudil for the treatment of neurodegenerative diseases. Neural Regen Res 17:2623-2631.

Xu J, Wen J, Fu L, Liao L, Zou Y, Zhang J, Deng J, Zhang H, Liu J, Wang X, Zuo D, Guo J (2021) Macrophage-specific RhoA knockout delays Wallerian degeneration after peripheral nerve injury in mice. J Neuroinflammation 18:234.

Xue W, Kong Y, Abu R, Roy P, Huh SH, Kuss M, Kumar V, Duan B (2022) Regulation of Schwann cell and DRG neurite behaviors within decellularized peripheral nerve matrix. ACS Appl Mater Interfaces 14:8693-8704.

Zhang M, Li C, Liu SY, Zhang FS, Zhang PX (2022) An electroencephalography-based human-machine interface combined with contralateral C7 transfer in the treatment of brachial plexus injury. Neural Regen Res 17:2600-2605.

Zhang W, Fang XX, Li QC, Pi W, Han N (2023) Reduced graphene oxide-embedded nerve conduits loaded with bone marrow mesenchymal stem cell-derived extracellular vesicles promote peripheral nerve regeneration. Neural Regen Res 18:200-206.

Zhao J, Wang X, Meng X, Zou W, Xu S (2021) Rapid and efficient wounding for in vivo studies of neuronal dendrite regeneration and degeneration. J Genet Genomics 48:163-166.

Zhong K, Huang Y, Zilundu PLM, Wang Y, Zhou Y, Yu G, Fu R, Chung SK, Tang Y, Cheng X, Zhou L (2022) Motor neuron survival is associated with reduced neuroinflammation and increased autophagy after brachial plexus avulsion injury in aldose reductase-deficient mice. J Neuroinflammation 19:271.

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