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Rapid injection of lumbar dorsal root ganglia under direct vision: relevant anatomy, protocol, and behaviors

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Author contribution statement

Xiaoman Yuan conceived the research, carried out the model building, coordinated the lab work and drafted the manuscript. Siyi Han and Fengtian Zhao performed the statistical analysis and drafted the manuscript. And Anne Many and Feng Gao took care of the behavior tests and drafted the manuscript. Jie Wang, Wen Zhang and Xuebi Tian participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Keywords

DRG injection, dorsal root ganglia, intraganglionic injections, neuropathic pain, Chronic Pain, SNI

Abstract

Word count: 200

Dorsal root ganglia (DRG) are anatomically well-defined structures that contain all primary sensory neurons and are distension nodules of the dorsal root in the spinal cord near the medial surface of each foramen. Therefore, DRG is considered to be a desirable target for injection to manage chronic pain. But it presents a limitation in probing deep into it without in vivo injection technology. Here, we described a technique for administering intraganglionic injections of lumbar DRG under direct vision. We use partial osteotomy rather than laminectomy, which removes more bone, to preserve spinal structures while gaining adequate DRG access. To monitor the intraoperative progress of the DRG injection, a non-toxic dye was utilized. The effectiveness of the injection on the diffusion of AAV within the ganglion was assessed by histopathology at postoperative day 21. Behavioral tests showed that neither motor nor sensory abilities were affected by saline or AAV injections. Meanwhile, the decreased pain threshold of SNI was considerably restored by pharmacological inhibition of DRG neurons. Our research achieved a more minimally invasive and intuitive intra-ganglionic injection in mice without damage to the spinal cord. In addition, the current methodology could be a valuable way that supports DRG injection preclinical investigations.

Contribution to the field

The DRG is a crucial structure in sensory transduction and modulation and DRG neurons have been considered as a significant target to study sensory nerve formation, function, and regeneration to aid in drug discovery and to elucidate mechanisms of peripheral nerve diseases. But the lack of in vivo minimally invasive injection technology presents a limitation in probing deep into it. Here, we described a method for conducting intraganglionic injections of lumbar DRG under direct vision. Previously, we observed the roles of neuropathic pain in neurological diseases. DRG is the first target for sensory nervous system disorders associated with pain due to its crucial role in sensory transduction. It is significant because laminectomy routinely used results in instability of the spinal cord with sensory and motor dysfunction, and the method we describe expected to be a facilitate new strategies for learning and treating chronic pain and other neuropathic disorders. We have achieved a new in vivo minimally invasive injection of DRG. Intra-DRG injection was not associated to Motor and sensory disfunctions. Therefore, this in vivo injection of DRG under direct vision provide an effective method for the further exploration of the nervous system.

Ethics statements

Studies involving animal subjects

Generated Statement: The animal study was reviewed and approved by Experimental Animal Care and Use Committee of Tongji Medical College, Huazhong University of Science and Technology.

Studies involving human subjects

Generated Statement: No human studies are presented in this manuscript.

Inclusion of identifiable human data

Generated Statement: No potentially identifiable human images or data is presented in this study.

Data availability statement

Generated Statement: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

In review

1 **Rapid injection of lumbar dorsal root ganglia under direct**
2 **vision: relevant anatomy, protocol, and behaviors**

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15

16 **Keywords:** dorsal root ganglia¹, DRG injection²,intraganglionic injections³, partial
17 osteotomy⁴ ,neuropathic pain⁵, chronic pain⁶,SNI⁷.

18 **Abstract:**

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^a Wen Zhang and Xuebi Tian contributed equally to this work

1 sensory neurons and are distension nodules of the dorsal root in the spinal cord near the medial
2 surface of each foramen. Therefore, DRG is considered to be a desirable target for injection to
3 manage chronic pain. But it presents a limitation in probing deep into it without in vivo injection
4 technology. Here, we described a technique for administering intraganglionic injections of
5 lumbar DRG under direct vision. We use partial osteotomy rather than laminectomy, which
6 removes more bone, to preserve spinal structures while gaining adequate DRG access. To
7 monitor the intraoperative progress of the DRG injection, a non-toxic dye was utilized. The
8 effectiveness of the injection on the diffusion of AAV within the ganglion was assessed by
9 histopathology at postoperative day 21. Behavioral tests showed that neither motor nor sensory
10 abilities were affected by saline or AAV injections. Meanwhile, the decreased pain threshold of
11 SNI was considerably restored by pharmacological inhibition of DRG neurons. **Our research**
12 **achieved a new minimally invasive and intuitive intra-ganglionic injection in mice.** In addition,
13 the present protocol may serve as a valuable resource for planning preclinical studies of DRG
14 injection.

15 **Introduction:**

16 The dorsal root ganglion (DRG) is a bilateral structure that is bundle-shaped, entering dorsally
17 from the **spinal cord** symmetrically from left to right within fixed bony vertebral structures
18 (neuroforamen)(Esposito MF et al., 2019;Terashima T et al., 2009)(fig1a,b). At the segmental
19 levels that innervate the limbs, each DRG has up to 15,000 neurons.(Nascimento AI et al.,
20 2018).The DRG, which is an extension of the dorsal root, is where primary sensory neurons'
21 somata are situated. (Devor M, 1999). The DRG is an essential structure for modulating and
22 transducing sensory information, including the transmission of pain. (Esposito M et al., 2019).

1 Nerve injury-related pain is typically persistent and difficult to treat.(Davis G and Curtin CM,
2 2016), and physiological modifications in the spinal cord and brain activity occur along with
3 neuropathic pain.(Widerström-Noga E, 2017). Meanwhile, numerous studies have
4 demonstrated that damage to the primary sensory neurons and their somata in the DRG are
5 significant sites for the development of pain (Berta T et al., 2017;Hu L et al., 2022;Martinello
6 K et al., 2022). The DRG exhibits notable phenotypic and functional changes after injury, and
7 these plastic alterations in the DRG as the primary source of pain signals delivered to the
8 brain(Berta T et al., 2017).Additionally, rodent DRG neurons have been used to investigate
9 sensory nerve development, regeneration, and function to support medical research and
10 explain the mechanism of peripheral nerve disorders such Charcot-Marie-Tooth disease and
11 diabetic neuropathy.(Noguchi K et al., 2004;Wang K et al., 2021) .

12 Direct injections into the DRG offer the chance to alter sensory neuron function at a
13 segmental level in order to investigate the pathophysiology of nociceptive diseases.

14 Intrathecal injection has been a widespread way to deliver medications to sensory neurons in
15 recent years, but it is impossible to confine the solution to a certain longitudinal length on one
16 side or at the vertebral level.(Fowler MJ et al., 2020;Kim H et al., 2020). According to Moore
17 et al.' research, agents can be injected into peripheral nerves to reach the DRG, but it could
18 injure the spinal cord and surrounding nerves and induce the agents to spread. (Moore DC et
19 al., 1954;Turner PV et al., 2011). Manipulating the neuron of one or more DRGs can increase
20 therapeutic effectiveness while minimizing side effects and avoiding the negative effects of
21 systemic medications(Atmaramani R et al., 2021;Nascimento AI et al., 2018). Laminotomy is
22 currently the most popular technique for DRG injection with direct access to the DRG, but

1 this may damage a segment of the spinal nerve and the DRG(Unger MD et al., 2017).
2 Furthermore, due to the absence of vertebral plate, the impact on spinal motor function and
3 the consequent secondary injuries are inevitable (Massie JB et al., 2004).
4 Here, we describe an easy and rapid microinjection technique that enables direct injection of
5 the agents into the DRG under direct observation. We remove the inferior articular eminence
6 of the upper conus and the superior articular eminence of the next conus at the injection site,
7 determine the minimum extent and best execution of the partial bone removal approach, and
8 directly expose the DRG at the target location for local microinjection. At the same time, we
9 tested the stability of this injection approach using dye and viral staining, the DRG could be
10 targeted without leaking. Additionally, we observed that blocking DRG neurons substantially
11 reversed the SNI mice's decreased pain threshold. Our study revealed a safe and effective
12 microinjection method that, combined with neuroscience techniques, may provide a new
13 intervention for the clinical treatment of neurological disorders.

14 **Materials and Methods:**

15 1. Animal subjects

16 Male C57 (6–8 weeks, 20-25g) mice were supplied from Tongji Hospital, Tongji Medical
17 College, Huazhong University of Science and Technology, Wuhan, Hubei, China. All animals
18 were raised under controlled conditions (22–25 °C, 12-hour alternate circadian rhythm, free
19 access to food and water, 3-4 mice per cage). All experiments received approval from the
20 Experimental Animal Care and Use Committee of Tongji Medical College, Huazhong
21 University of Science and Technology, and were in agreement with the National Institutes of
22 Health Guidelines for the Care and Use of Laboratory Animals.

1 2.Injection

2 The DRG injection was performed using a prone position anesthetized with
3 pentobarbital(fig3). The skin was shaved and disinfected, and a longitudinal, approximately 1
4 cm incision was made over the middle above the posterior superior iliac spine to expose the
5 subcutaneous muscles(fig3a). Identify the first junction of the spinous process and silvery
6 white aponeurosis as the location marker (L1 spinous process)(fig3c. A muscle incision is
7 made immediately adjacent to the spinous process on the left side and the paravertebral
8 muscles are bluntly separated to expose the cone, where the left intervertebral joint and left
9 vertebral plate are visible(fig3 d,e,f,g)). The DRG is located below the intervertebral joint
10 when the mouse is in prone position. The inferior articular eminence was amputated with a
11 cranial drill and ophthalmic scissors along the line from the lumbar to the inferior edge of the
12 previous cone(fig3h), and the superior articular eminence of the next cone was intercepted in
13 a horizontal direction(fig3i). The accessory process is visible after the osteotomy, and the
14 DRG is in its axilla(fig3j,k,l). The surrounding connective tissue is carefully and bluntly
15 separated .

16 All injections were performed using a microprocessor-controlled injection system employing
17 a stereotaxic holder (Item: 68030, RWD, Shenzhen, China), equipped with a pulled glass
18 capillary injection tip(fig3m,n). Fine tune the needle path until the needle lumen was aligned
19 with the center of the DRG and moved vertically downward until the tip begins to retract into
20 the DRG. The tip was advanced further in small increments until the tip pierced the DRG (the
21 surface depression of the DRG was restored at the moment). Slowly and gradually withdraw
22 so that the tip was submersed in the three-dimensional center of the DRG. A quantity of agent

1 (dye, saline or AAV) was injected through a calibrated glass microelectrode connected to an
2 infusion pump (Hamilton CMA 400) at a rate of 20 nl/s. At the end of the injection, the pipette
3 was left at the injection site for 5 min to avoid virus. Finally, the incision was sutured and
4 disinfected with iodophor.

5 3. Induction of neuropathic pain

6 The neuropathic pain was performed according to the procedures previously described (Xiong
7 B et al., 2020). After a mouse was given a pentobarbital sodium anesthetic (50 mg/kg,
8 intraperitoneally), the left sciatic nerve, which has three branches (the common peroneal,
9 tibial, and sural nerves), was visible. The common peroneal nerve and the tibial nerve, the two
10 branches of the sciatic nerve, were sectioned distally to the ligation and tightly bound with a
11 5.0 silk thread (or suture), removing 2 to 4 mm of the distal nerve stump. The intact sural
12 nerve was carefully avoided by preventing any contact or tension. The skin was then shut.

13 4. Basso-Beattie-Bresnahan (BBB) motor function scores

14 BBB score was used to evaluate hindlimb motor function (Dergham P et al., 2002). Mice were
15 placed on a circular platform with a diameter of 2 m. Scores for hindlimb walking and limb
16 activity were calculated and evaluated. In the initial stage, the hindlimb joint activity received
17 a score (0–7 points). The second stage (8–13 points) evaluated gait and coordination of the
18 hindlimbs. The third level (14–21 points) assessed the minute paw motions made while
19 moving. The three phases combined for a total score of 21. We performed the BBB on day 0,
20 1, 3, 7, 14, and 21 after surgery.

21 5. Punctate mechanical stimulation (von Frey)

1 The mechanical paw withdrawal threshold (MPWT) of the ipsilateral hind paw was
2 determined using Von Frey filament, which simulated the mechanical allodynia as previously
3 described(Bonin RP et al., 2014). All behavioral evaluations were performed from 8:30 am to
4 4:30 pm. Briefly, mice were placed into individual plastic containers on a metal mesh floor
5 and acclimated to their environment for 30 minutes. Positive responses included shaking,
6 licking, and rapid paw withdrawal. The minimum amount of force required to elicit a positive
7 response or MPWT was assessed as previously discussed (in grams). An independent
8 researcher who was unaware of the study design performed all behavioral tests.

9 6.Open-field test (OFT)

10 The OFT was used to evaluate the exploratory locomotor ability of mice. Mice were placed in
11 the center of a grey polyethylene box (50 × 50 × 40 cm) and allowed to explore freely for 5
12 min. An automatic video tracking system (AVTAS v3.3; AniLab Software and Instruments
13 Co., Ltd., Ningbo, China). Between each test, the surface of the arena was cleaned with 75%
14 alcohol to avoid the appearance of olfactory cues.

15 7.Immunofluorescence staining

16 The spinal cord and DRG tissues were taken after sodium pentobarbital anesthesia (50 mg/kg,
17 intraperitoneal) in mice, which perfused intracardially with saline followed by 4% ice-cold
18 paraformaldehyde in 0.1 M phosphate-buffered saline(Sleigh JN et al., 2016). The samples
19 were then dehydrated in a 30% sucrose solution overnight at 4 °C after being postfixed in 4
20 percent paraformaldehyde for 4 hours at 4 °C. Using a cryostat microtome(Thermo Fisher,
21 NX50, Waltham, MA), the fixed spinal cord was cut into 20 μm thick coronal slices. Sections
22 were stained for 10 minutes at room temperature with DAPI, followed by a PBS wash (3

1 times, 10 min each). Finally, a virtual microscope slide scanning system (Olympus, VS 120,
2 Tokyo, Japan) was used to see the immunostained sections. Photographs of sections
3 containing the region of interest (ROI) were cropped in ImageJ (National Institutes of Health,
4 Bethesda, MD). The quantity of cells and neurons in the DRG were measured with ImageJ .
5 The DAPI stained nucleus was counted as total cells .

6 8. Virus Injections and chemogenetics

7 Briefly, mice were anesthetized with sodium pentobarbital anesthesia (50 mg/kg,
8 intraperitoneal). For cell-type-specific in situ labelling, 200 nl rAAV-EF1 α -EGFP-WPRE-pA
9 was injected with the L4 DRG and detected by expression staining 3 weeks after AAV
10 expression. For chemical inhibition, we used the virus rAAV-hSyn-hM4D(Gi)-mCherry to
11 inhibit neuronal activity in the DRG. Mice were injected 200 nl of rAAV-hSyn-hM4D(Gi)-
12 mCherry into the DRG. SNI surgery was performed 7 days after AAV virus injection. 14 days
13 later, mice were given von Frey tests before and 1 hour after CNO(Clozapine N-oxide, a
14 human muscarinic designed receptor agonist) (5 mg/kg) administration intraperitoneally. The
15 MPWT was measured 1 day after the CNO injection. Then, mice were perfused to examine
16 viral infection in the DRG.

17 9. Experimental designs treatment

18 The design of this study is shown in Figure 4a, 5a and 6a. First, we examined the
19 intraoperative progress of the DRG injection with a non-toxic dye. Then, AAV was used to
20 determine the success of injection. Meanwhile, three dosing regimens were also designed for
21 the control, saline (200nl), and AAV (200nl) groups to explore the effects of the injection

1 operation on movement and sensation. Finally, chemogenetic approach was used to inhibit
2 DRG neurons(Farrell MS and Roth BL, 2013). CNO (Sigma-Aldrich, dissolved in 0.1%
3 DMSO in saline 5mg/kg) or saline was injected intraperitoneally after 3 weeks of injection of
4 AAV- hM4Di at the end of the virus incubation period.

5 10.Statistical analysis

6 All results are shown as mean \pm SEM.In analyses, an unpaired Student's t-test was applied for
7 comparing two groups. Two-way ANOVA was used for group comparisons, followed by the
8 Bonferroni post hoc test. Applying Pearson coefficients allowed us to statistically convey
9 relevance. GraphPad Prism 7.0 was used for statistical analysis, and $P < 0.05$ was considered
10 statistically significant in this study.

11 **Results:**

12 1. Partial bone removal allowed good exposure of the DRG and injection

13 The anatomical location of the lumbar lateral dorsal root ganglion is under the facet joint and
14 under the transverse axilla when the mice were in prone position (fig1). The DRG was
15 exposed by removing the upper and lower articular processes corresponding to the target
16 DRG, as shown in fig2. The DRG under the transverse axillary fossa after partial osteotomy
17 could be seen directly through the microscope (fig3). The drawn glass capillary was adjusted
18 above the target DRG, and the height of the capillary was adjusted vertically down through
19 the DRG and successfully submerged under direct vision. Afterwards, 1 μ l of dye was
20 injected directly into the DRG, which showed no extra-DRG dye leakage and no DRG rupture
21 or damage under microscopy (fig3h,i).

22 2. Conventional doses of virus were injected into the DRG without leakage

1 To detect the modulation of neuronal function in the target DRG using this method can be
2 achieved with less damage. We injected AAV virus into the target DRG and tested the viral
3 expression after 3 weeks of viral transfection (fig4a). The results of immunofluorescence
4 showed that the virus was expressed only in the ipsilateral DRG and did not spread to the
5 contralateral and spinal cord (fig4b). And the rate of positive cells, as a percentage of all cells,
6 is $2.3516233\% \pm 0.3783797\%$ (n = 3 mice).

7 3. Motor and sensory functions were not affected after virus injection into the DRG of mice
8 MPWT was used to detect mechanical allodynia. BBB and FCT were performed to evaluate
9 the motor function after virus injection. The behavioral tests were performed on days 0, 1, 3,
10 7, 14, and 21 (fig5a). Compared to the control group, there was no significant difference in
11 MPWT after injection of saline or AAV (fig5b, group: $F(2, 72) = 0.2632$, $P = 0.7694$; time: $F(5, 72) = 0.2695$,
12 $P = 0.9284$; interaction: $F(10, 72) = 0.8821$, $P = 0.5538$). BBB score showed
13 that there was no decrease in motor ability of hind limb after the operation (fig5c, group: $F(2,$
14 $72) = 0.5652$, $P = 0.5707$; time: $F(5, 72) = 0.5652$, $P = 0.7263$; interaction: $F(10, 72) =$
15 0.09565 , $P = 0.9998$). In OFT, no significant difference was found in the distance (fig5d,
16 group: $F(2, 72) = 0.741$, $P = 0.4802$; time: $F(5, 72) = 1.121$, $P = 0.3573$; interaction: $F(10, 72)$
17 $= 0.5286$, $P = 0.8645$) and max speed (fig5e, group: $F(2, 72) = 0.2902$, $P = 0.7490$; time: $F(5,$
18 $72) = 0.8515$, $P = 0.5180$; interaction: $F(10, 72) = 0.2137$, $P = 0.9943$) among the control,
19 saline and AAV groups. These results suggest that there was no dysfunction of motor or
20 sensory after injection in long or short periods.

21 4. Application of chemical inhibitors in DRG effectively inhibited hyperalgesia induced by
22 SNI

1 A pharmacologic inhibitor of the virus CNO was used to test whether selective inhibition of
2 mice DRG neurons suppressed SNI-induced behavioral responses (fig6a). The results of
3 immunofluorescence showed that AAV-hM4Di injection was expressed in the DRG and the
4 rate of positive cells is $3.8515644\% \pm 1.8687406\%$ (fig6b, n = 3 mice). Notably, the
5 mechanical allodynia induced by SNI was reversed on day 14 after administration of
6 CNO](fig6c, t = 6.248, P < 0.0001). However, compare to the saline group, there was no
7 significant difference in MPWT 1 day after administration of CNO (fig6c, t = 0.1116, P >
8 0.9999). These results further support that local injection of DRG modulated the development
9 of chronic pain.

10 **Discussion:**

11 The present study has defined a method for selective injection of drugs or viruses into the
12 target DRG. Our research achieved a new minimally invasive and intuitive intra-ganglionic
13 injection in mice. Intra-DRG injection does not cause sensory or motor function. In addition,
14 injection of chemical inhibitors in L4 DRG of SNI mice effectively alleviated mechanical
15 hypersensitivity caused by surgery, which confirmed that our improved technique offers
16 additional possibilities for studying sensory mechanisms and treating chronic neuropathic
17 pain.

18 Various methods have been used to deliver agents to the DRG for application
19 study(Schultheis B et al., 2021). Although the basic method of alternative injection sites for
20 DRG treatment, intrathecal injection by lumbar puncture, is simple to perform, agent diffusion
21 is unavoidable due to the high doses of medications utilized. (Lyu YS et al., 2000;Xu JJ et al.,
22 2006). In contrast to direct injection into the DRG, sciatic nerve injection harms peripheral

1 nerves and targets numerous DRGs.(Jung Kim H and Hyun Park S, 2014). Due to the severe
2 vertebra impairments, direct injection laminectomy causes spinal cord instability and sensory
3 and motor dysfunction.(Papagelopoulos PJ et al., 1997). Partial laminectomy mentioned by
4 Gregory Fischer requires a shallow angle of incidence to the nerve surface(Fischer G et al.,
5 2011). Our research had several benefits for the delivery of agents to the DRG. First, injection
6 is carried out utilizing a Hamilton syringe and a Small Animal Stereotactic Frame with the
7 Microinjection Adaptor, potentially causing less damage to the DRG and offering a solid
8 platform for injection. Second, a moderate amount of bone removal maintains the spine's
9 stiffness, allowing to better preserve neurological function and improving the accuracy and
10 repeatability of following studies by preventing further damage from spinal instability in the
11 late postoperative period. Thirdly, after removal of the articular eminence, the DRG is located
12 in the axilla of the anapophysis which attached to a silver-white tendon. This simple
13 dissection procedure can be easily completed in 20 – 30 minutes per mice due to the clear
14 positioning markings, from animal anesthesia to injection. Finally, there is no need to adjust
15 the angle of injection, and direct vision under the microscope reduces the risk of missing the
16 target and completely penetrate the DRG and reach the ventral root. Meanwhile, small
17 incisions were required under direct vision to avoid hyperalgesia induced by surgical
18 exposure of the ganglion.

19 DRG-targeted injections may serve as an important means of modulating sensory funct
20 ion(Terashima T et al., 2009), so it is critical to ascertain the effect of the injection. I
21 n contrast to the control group, our data showed that there was no hyperalgesia follo
22 wing surgical exposure and ganglion injection. DRG segmental injection has a potentia

1 future in the field of viral vector-mediated gene therapy. Our findings demonstrated that by directing DRG injections to modulate neuronal activity, significant transduction can be accomplished. The use of neuron-specific viruses in our study could achieve an infection rate (The proportion of positive cells in the total number of cells) of approximately 2% (rAAV-EF1 α -EGFP-WPRE-pA) and 4% (rAAV-hSyn-hM4D(Gi)-mCherry). Mecklenburg's research estimated that the overall percentage of sensory neurons among all mice DRG cells is about 3-5% (Keeler AB et al., 2022; Mecklenburg J et al., 2020). And in the current study, we discovered that in SNI rats, inhibiting the DRG neurons can significantly reduce mechanical allodynia. The mechanical allodynia caused by SNI was shown to be effectively reduced by inhibiting the DRG neuronal activity, but the relief was only temporary. This may be because CNO binds to hM4Di to hyperpolarize the cell membrane and inhibit the action potential of neurons, and CNO typically takes two hours to clear from the membrane after being applied to the neuron. (Roth BL, 2016). Thus, by combining anatomically selective drug delivery mechanisms with novel molecularly selective drugs, along with other neuroscientific tools, this protocol may promote new strategies for treating chronic pain. The dissection technique described in this study does have its limitations. Firstly, the method is primarily applicable to the lumbar DRGs and is unlikely to be applied at other places. Secondly, reinjection is impossible, but the use of indwelling tubes within the DRG could be considered. Finally, The advantages of this strategy over other ways need to be further investigated because our study did not compare it to previous ones. However, This option would sup

1 port preclinical studies of injectable solutions administered intralaryngeally to advance
2 previous findings in rodent studies.

3 **Conclusion**

4 Techniques for in vivo injection can make it easier to explain the correlation between
5 behaviour and underlying mechanism. Our research provided a quick, efficient, and visible
6 method for single intra-DRG injection. The results of the current investigation demonstrated
7 that no motor or sensory deficits developed throughout the injection procedure. Chemical
8 inhibition of DRG neuron alleviated hyperpathia induced by SNI. Therefore, DRG may be a
9 promising target for neuropathic pain researches, and in vivo injection of DRG under direct
10 vision provide an effective method for the further exploration of the nervous system.

11 **List of abbreviations**

12 DRG: dorsal root ganglion; SNI: spared nerve injury; MPWT: The mechanical paw
13 withdrawal threshold. CNO: clozapine-N-oxide

14 **Ethics approval and consent to participate**

15 All experiments were approved by the Experimental Animal Care and Use Committee of
16 Tongji Medical College, Huazhong University of Science and Technology, and were in
17 agreement with the National Institutes of Health Guidelines for the Care and Use of
18 Laboratory Animals.

19 **Consent for publication**

20 Not applicable.

21 **Availability of data and materials**

1 The data and materials supporting the conclusions of this study are available from the
2 corresponding author on reasonable request.

3 **Competing interests**

4 The authors declare that they have no competing interests.

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9 **Authors' contributions**

10 Xiaoman Yuan conceived the research, carried out the model building, coordinated the lab
11 work and drafted the manuscript. Siyi Han and Fengtian Zhao performed the statistical
12 analysis and drafted the manuscript. And Anne Many and Feng Gao took care of the behavior
13 tests and drafted the manuscript. Jie Wang, Wen Zhang and Xuebi Tian participated in its
14 design and coordination and helped to draft the manuscript. All authors read and approved the
15 final manuscript.

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35

1 **Figures and legends:**

2

3 **Figure1. Peripheral anatomy of the DRG.** (a) The DRG is a cluster of sensory neuron cell
4 bodies located in the dorsal roots of the spine. The schematic portrays a transverse section of
5 the spine. (b) The bony structures surrounding the DRG (lumbar segments, anterior view). The
6 transverse process has been exaggerated to make it easier to understand. (c, d) Posterior and
7 left side view of spine. The DRG is seen in the foramina. It is difficult to see the transverse
8 processes in the posterior view due to the short transverse process in mice. The image depicts
9 a large one for better understanding.

10

11 **Figure2. Paravertebral surgical exposure for ganglionic injection.** Images show as the
12 operative field (top panel), cleaned vertebral bones (middle panel), and reference diagram
13 (bottom panel). (a, b) Preliminary soft tissue dissection at the level of the L4- L5 facet joint
14 shows the spinous processes (sp), inferior articular process(iap), and superior articular
15 processes (sap), as well as the lamina bone (lam) and accessory process(ap) on L4. The dorsal
16 root ganglia are covered by a facet joint. (c) Removal of the facet joint bone above the foramen
17 reveals the distal dorsal ganglion, which is much larger in diameter and located under the arm
18 of L4 accessory process.

19

20 **Figure3. Procedure of DRG injection under direct vision.** (a) After anesthesia, a small
21 incision is made in the dorsal skin at the level superior to posterior superior iliac spine. (b) The
22 incision is enlarged for better visualization (for display only, not part of the operation steps). (c)
23 The first junction of the spinous process and the silvery white aponeurosis is identified as a
24 positional marker (L1 spinous process). (d) A deep muscle incision is made along the lateral
25 surface of the spinous process. (e-g) Expose the left intervertebral joint and the left vertebral
26 plate. (h) Excision of the inferior articular eminence. (i) Excision of the superior articular
27 eminence. (j) Exposure of the DRG. (The distal end is fixed with forceps because the display
28 causes the wound to widen, resulting in distal fixation failure). (k, l) Visual field during in vivo
29 injection. (The DRG is filled with dye). (m) Diagram of the experimental equipment set-up. (n)
30 Injection using a glass needle containing mineral oil and a trace of viral solution. ap, accessory
31 process.

32 **Figure4. Virus expression 21 days after the DRG injection.** (a) AAV is injected at the L4
33 DRG. (b) The virus is expressed at the injection site, in the contralateral DRG and in the spinal

1 cord. The virus was confined to the DRG without leakage (n = 5 mice per group).

2

3 **Figure5. Changes in sensory and motor function after surgery and injection.** (a) The
4 schedule of administrating AAV or saline, BBB test, MPWT and OFT. (b) Compared to the
5 control group, no effect is observed on the sensory function after saline or AAV administration
6 ($P > 0.05$, n = 5 mice per group). (c) The scores of the control, saline and AAV groups are
7 similar at the corresponding time points ($P > 0.05$, n = 5 mice per group). The manipulation
8 had no effect on motor function. (d) In the OFT, there are no significant differences in the
9 distance and maximum velocity among the three groups ($P > 0.05$, n = 5 mice per group).

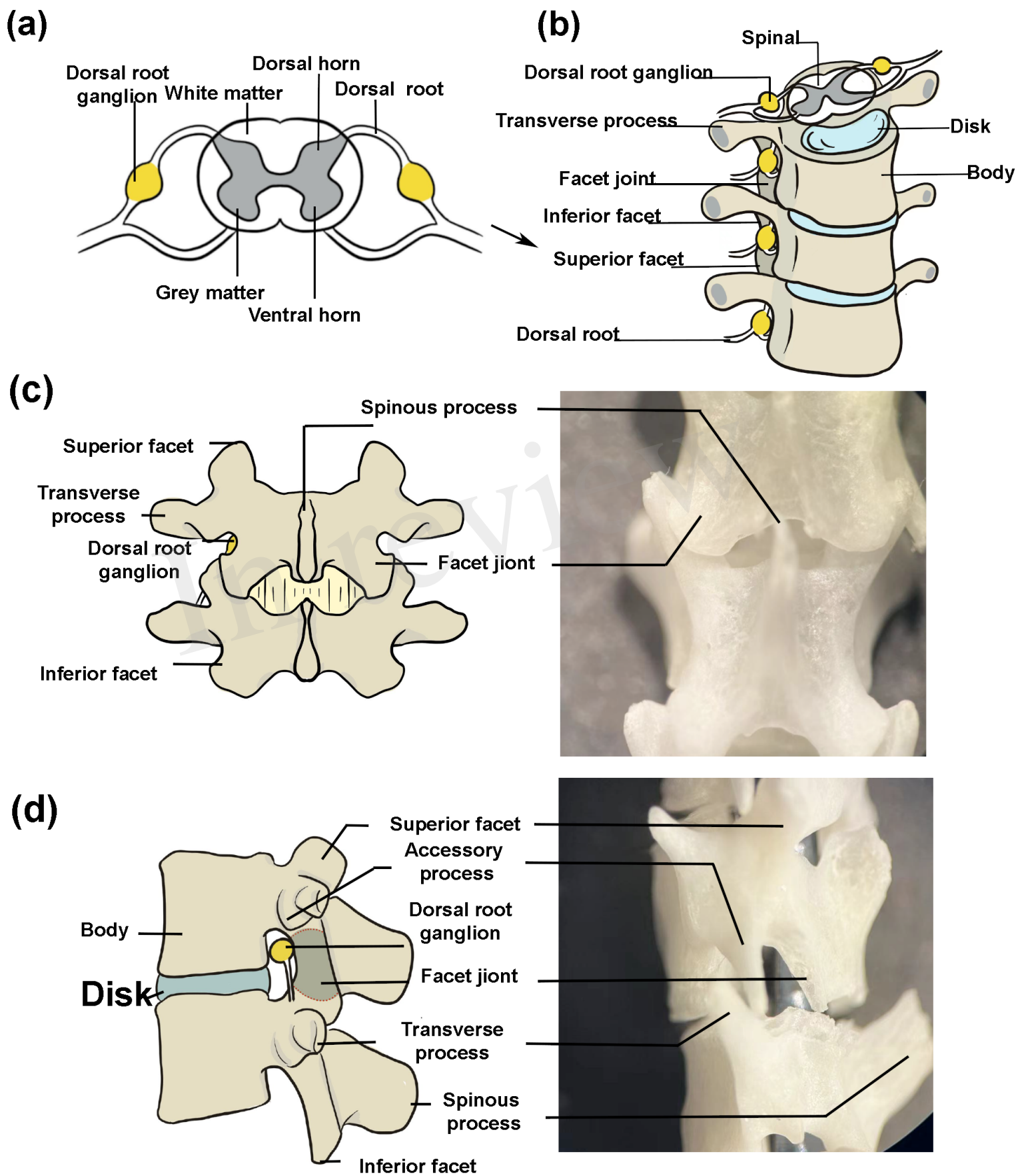
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11 **Figure6. DRG may serve as a target for chemogenetic reversal allodynia induced by SNI.**

12 (a) The schedule of administrating AAV, saline or CNO, MPWT. (b) AAV virus expressed in
13 the DRG (n = 5 mice per group). (c) Compared to the saline group, CNO effect reverse allodynia
14 induced by SNI (**** $P < 0.0001$, n = 5 mice per group), with effect disappearing after 1 day
15 ($P > 0.05$, n = 5 mice per group).

In review

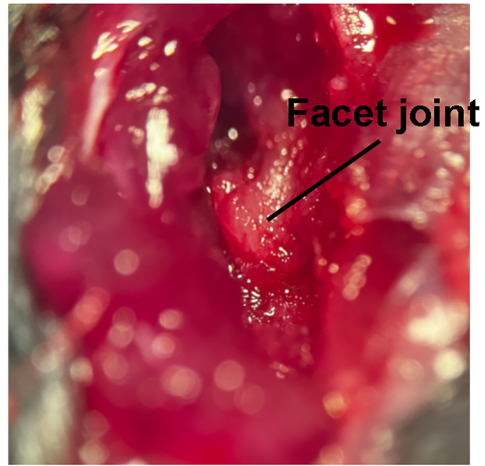
Figure 1.TIF



(a) joint exposure



(b) joint exposure



(c) partial laminectomy

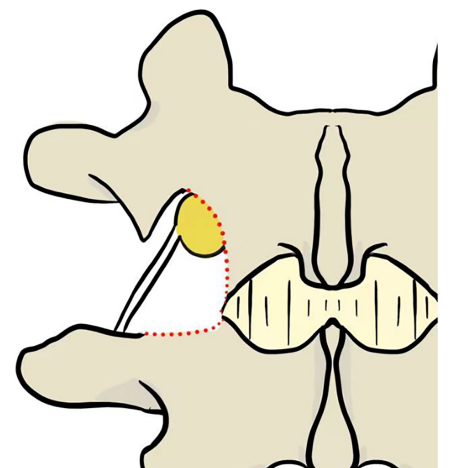
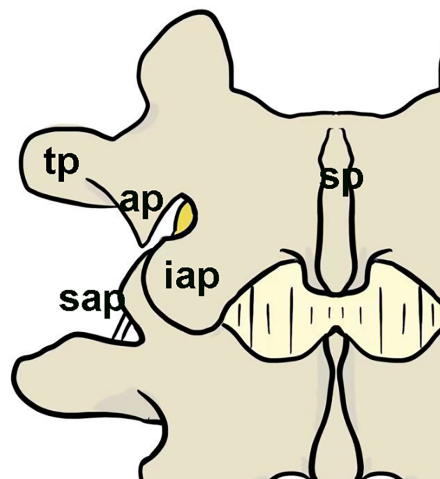
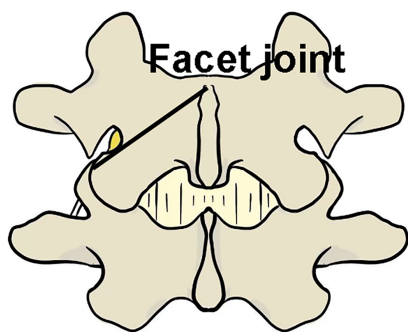
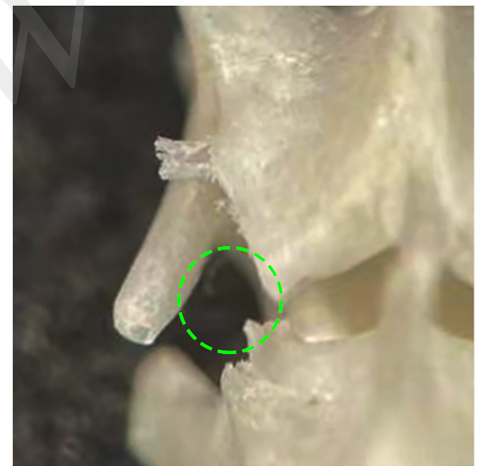
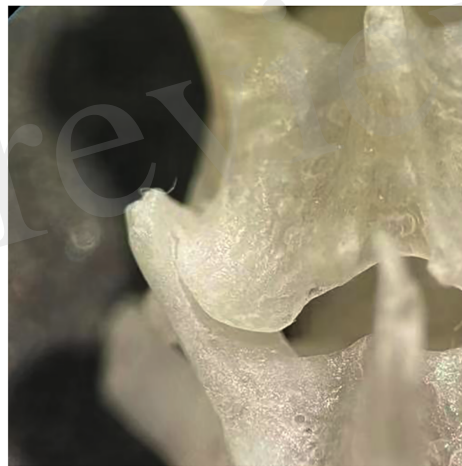
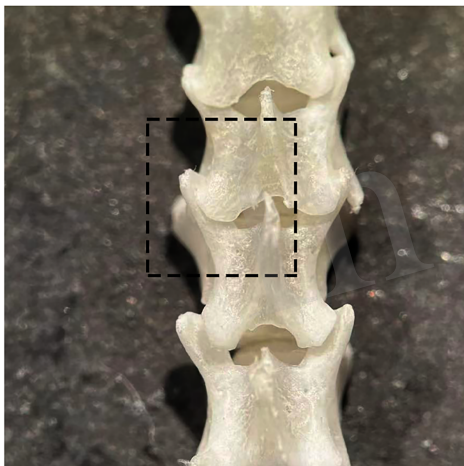
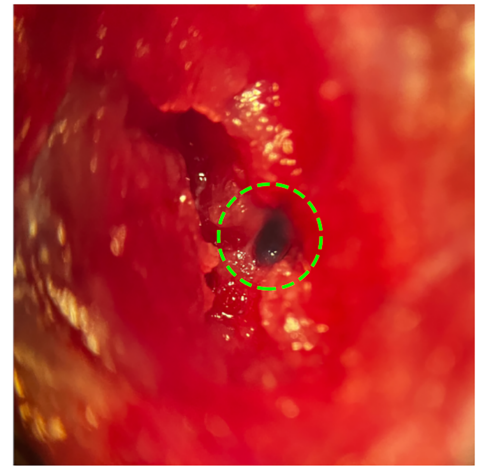


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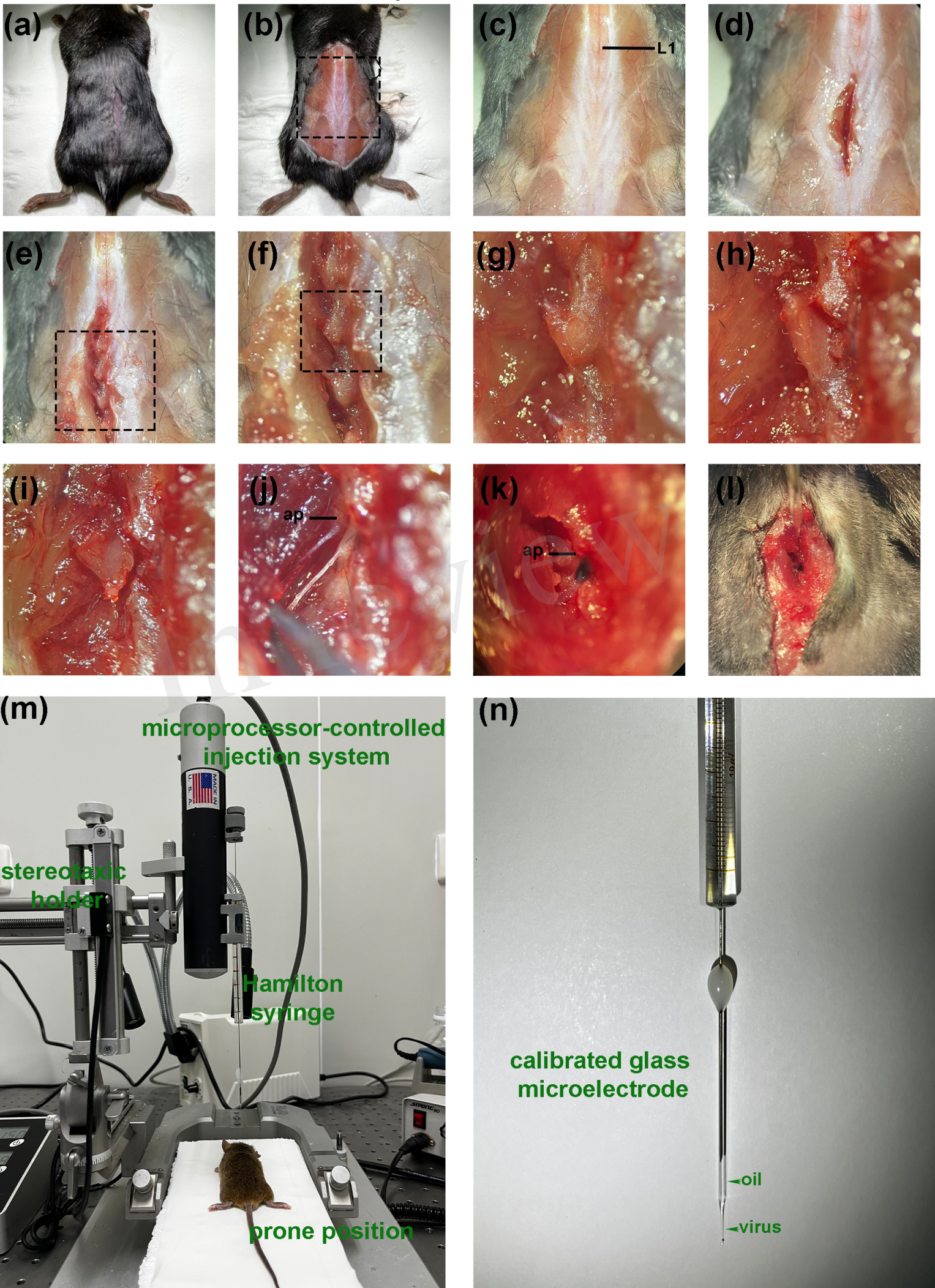


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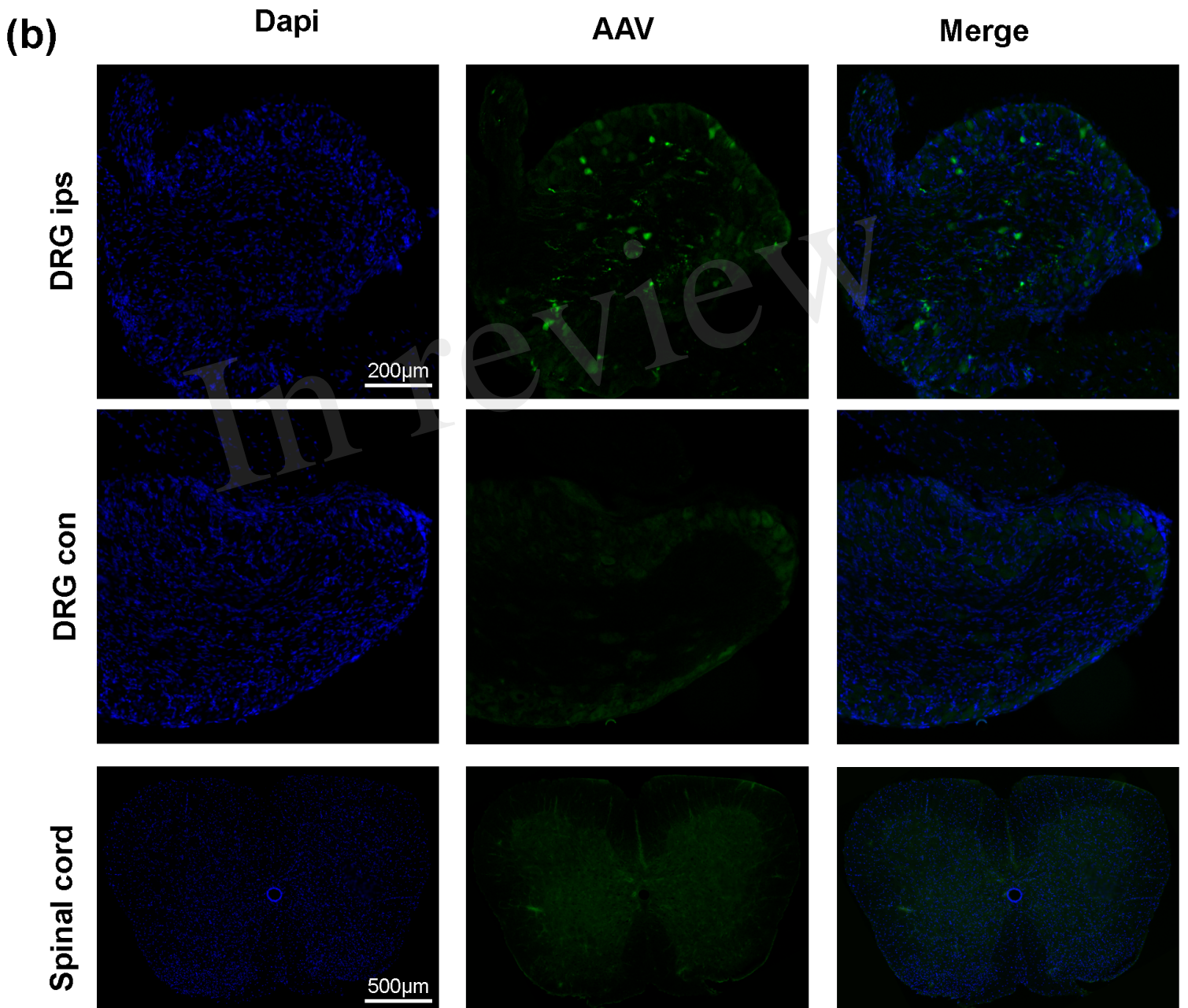
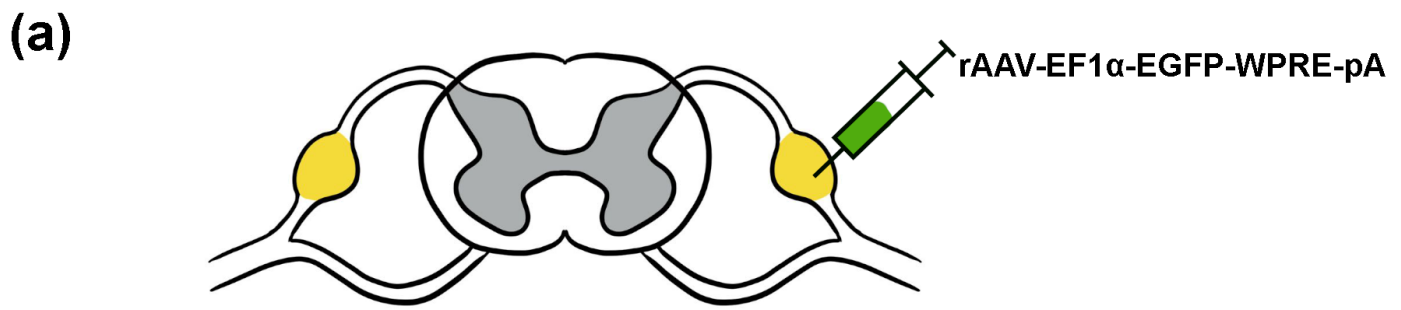
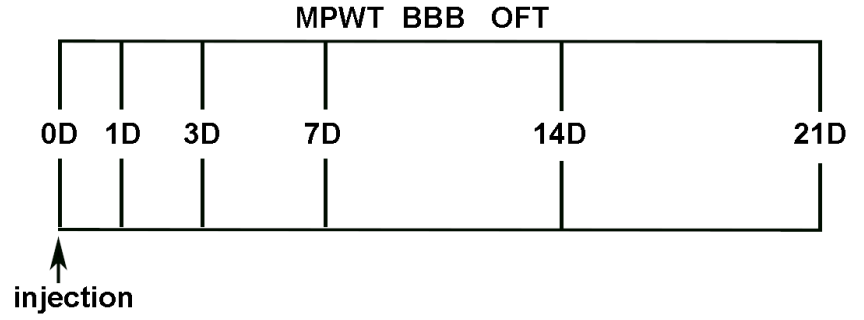
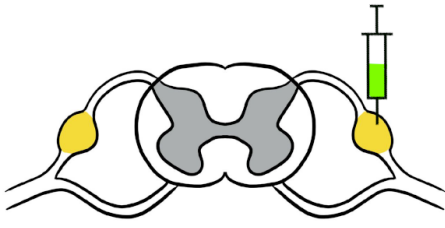


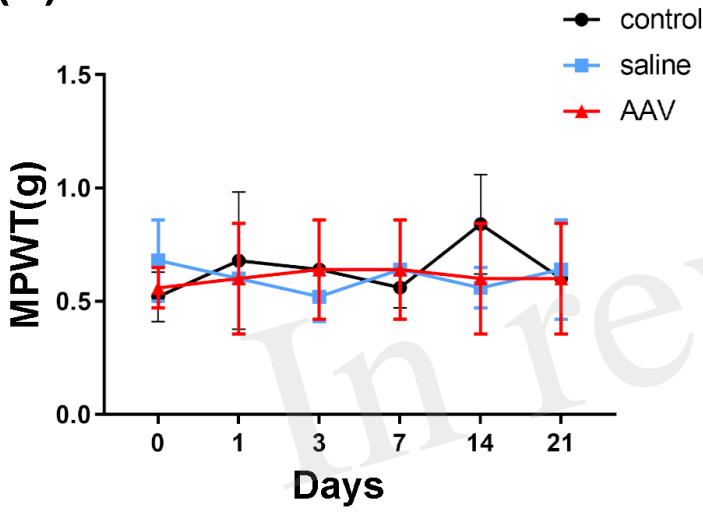
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(a)

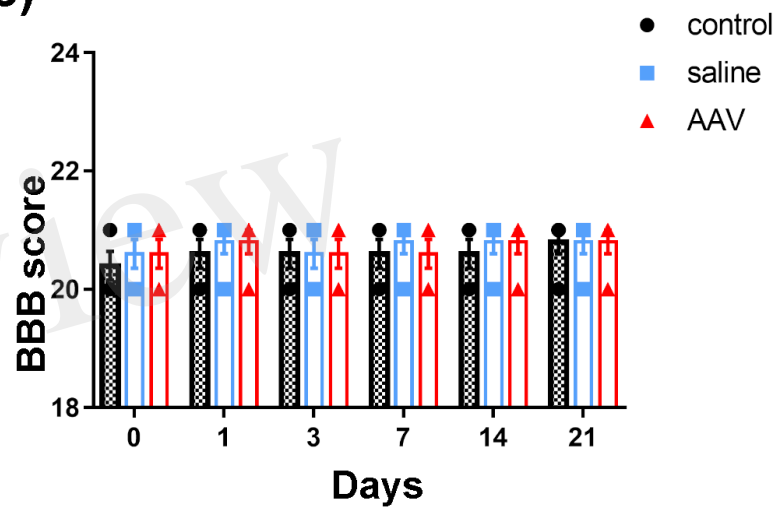
rAAV-EF1 α -EGFP-WPRE-pA/saline



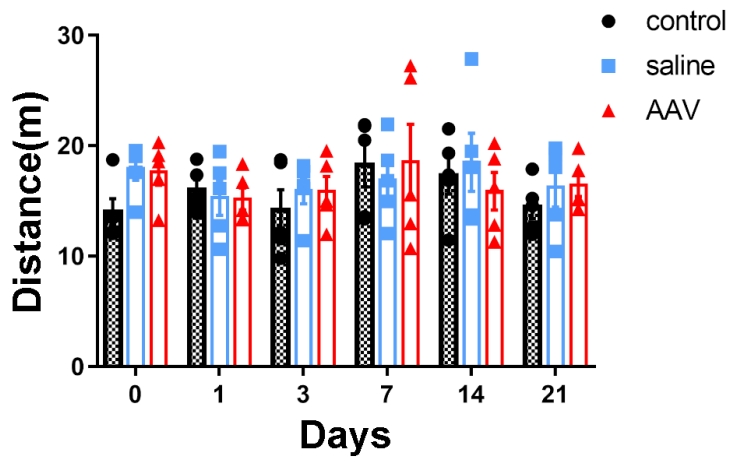
(b)



(c)



(d)



(e)

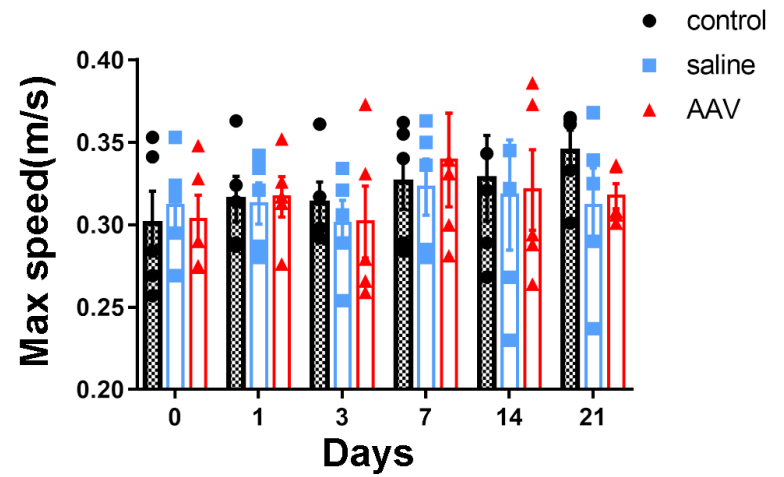


Figure 6.TIF

