

## Original Article

# Preparation of trigeminal neuralgia animal model through stereotactic trigeminal nerve compression technology

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**Abstract:** Background: The Trigeminal neuralgia incidence rate in China is about 182/100, 000. Given the lack of pathophysiological understanding of TN, the establishment of a stable and lasting TN animal model is essential to its further pathophysiological study. Methods: The preparation of trigeminal neuralgia (TN) animal model through stereotactic trigeminal nerve compression technology was investigated. Rats were randomly divided into four groups, namely, two experimental and two saline control groups. ION ligation and agar-compressed trigeminal nerve root (TNR) were applied to establish the TN rat model. Videos were revealed for the observation of free behaviour and responses to mechanical facial stimulation to analyse and assess the possible behavioural alterations that would indicate the establishment of TN. Results: After surgery, all experimental rats exhibited changes towards both non-evoked and evoked behaviour. Although CCI had significant changes, the intragroup individual differences were large. The group of agar-compressed TNR still exhibited significant changes 42 d after surgery. No large intragroup individual difference was found, but the operation was relatively difficult than the other method. Conclusions: The establishment of TN rat model by agar-compressed TNR was relatively stable, and could be applied to the pathophysiological study of TN.

**Keywords:** Animal model, neuropathic pain, trigeminal neuralgia, rat behavior, allodynia, compression

## Introduction

The Trigeminal neuralgia (TN) incidence rate in China is about 182/100, 000 [1]. Given the lack of pathophysiological understanding of TN, the establishment of a stable and lasting TN animal model is essential to its further pathophysiological study. Early, the animal model through chronic compression towards the peripheral nerve obtained wide acceptance [2-8]. However, clinical practices found that TN is more closely related to vascular compression on the trigeminal nerve root (TNR) [9-12]. Considering that the trigeminal nerve is the mixed nerve of motor and sensory nerves, the stereotactic positioning technology was used in this study. Under the guidance of a nerve stimulator, a TN model was established by simulating vascular compression through the injection of agar into the cerebellopontine angle (CPA) to compress TNR. A TN rat model with traditional

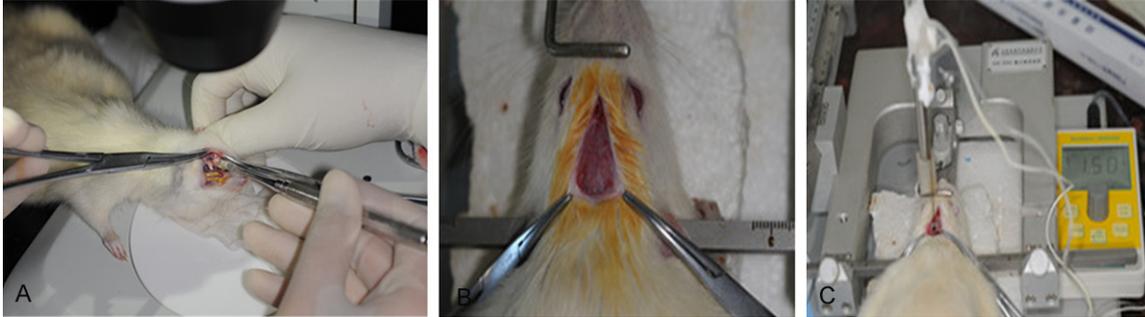
infraorbital nerve ligation was also established for behavioural comparison to evaluate the stability and reliability of the TN model.

## Materials and methods

### *Animals and grouping*

Forty healthy adult male Sprague-Dawley (SD) rats (with a weight of 250 g to 290 g), provided by the Experimental Animal Ministry, China Medical University, were randomly divided into four groups: T1 group, infraorbital nerve ligation group (n = 10); T2 group, agar-compressed TNR group (n = 10); C1 group, saline-injected infraorbital nerve control group (n = 10) and C2 group, saline-injected TNR control group (n = 10). Two SD rats were placed in a transparent plastic cage (35 cm × 25 cm × 15 cm) with 12 h cycle fluorescent lighting, and given adequate food and water. The room temperature ranged from

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**Figure 1.** Surgical procedures of the experimental operation. A: Exposure and separation of the infraorbital nerve; B, C: Stereotactic positioning of TNR; C1 group: the surgical procedures were the same as T1 group, while 4 ul saline was injected into the exposed left infraorbital nerve, then sutured the wound; C2 group: the surgical procedures were the same as T2 group, after the precise positioning of TNR, 10 ul saline was injected, and then sutured the wound.

**Table 1.** The scores of MSNT

	1	2	3	4	5	6	7	8	9	10
T1	4	3	4	4	3	3	4	4	4	3
C1	0	1	0	1	0	0	0	1	1	1
T2	4	4	4	4	3	4	4	4	3	4
C2	0	1	2	1	0	0	1	1	1	0

18°C to 21°C to avoid unnecessary irritation. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of China Medical University.

### Surgeries

The surgical environment of all groups was the same. In T1 group, a rat was intraperitoneally injected with 10% chloral hydrate (0.3 mL/kg) as anaesthesia. After anaesthesia, the rat was placed on the laboratory table at the supine position, and the left side was set for the surgery. After disinfection of the surgical area, a 1 cm incision was made along the palatum of the maxillary first molar. The mucoperiosteum was then separated to expose the infraorbital nerve. Two medical intestinal chrome lines (5.0) were used to ligate the infraorbital nerve, with a space of approximately 2 mm between the two ligation points. The tightness of the ligation was approximately -75%, which would reduce the diameter of infraorbital nerve for only 50%, and delay but not completely block the nerve conduction. Thus, the infraorbital blood flow could

still circulate. Finally, the incision was sutured using a 3-0 suture [8] (**Figure 1A**).

In T2 group, after anaesthesia, a rat was fixed in a stereotaxic instrument at the prone position (DW-2000, Taimeng Technology Co., Ltd., China), with the microsyringe fixed on the guide rod. After disinfection of the surgical area, the scalp of the rat was cut along the midline, and the periosteum was stripped. The TNR in the left CPA was positioned using stereotactic positioning technology, i.e., 7.5 mm towards the posterior bregma and 3.2 mm left to the midline [13]. The animal skull drill was applied for deossification, and the sense of bonelessness indicated the end of deossification. A plexus stimulator Multistim SENSON (Pajunk GmbH Medizin Technologie, Germany) was connected. The tissues were penetrated, and a current stimulation of 0.15 mA was initiated at the stereotactic-positioned TNR. The alarm of the nerve plexus stimulator proved the exact positioning of TNR. The precise puncture depth was then measured for guiding and adjusting the microsyringe needle to reach the precise point of TNR. An agar solution (4%; 10 µL) was then injected to the rat [13]. The syringe was fixed for 5 min for the solidification of agar. Then, the syringe was slowly pulled out. The incision was sutured using a 3-0 suture (**Figure 1B** and **1C**).

### Mechanical stimulation-nociceptive threshold (MSNT)

Given that no method can quantify the spontaneous pain of an animal, the appearance of retraction, escaping or aggressive behaviour, asymmetrical facial scraping and changes in

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**Table 2.** Changes of MSNT in different groups

Groups	Preoperatively	The postoperative 3 <sup>rd</sup> d	The postoperative 7 <sup>th</sup> d	The postoperative 14 <sup>th</sup> d	The postoperative 28 <sup>th</sup> d	The postoperative 35 <sup>th</sup> d	The postoperative 42 <sup>nd</sup> d
T1	13.20±0.51	13.5±0.78	2.77±0.56	0.99±0.21	4.34±0.36	9.21±0.72	12.70±0.34
T2	13.10±0.48	7.8±0.56	0.87±0.45	0.95±0.34	1.02±0.39	3.04±0.35	3.01±0.31
C1	13.47±0.34	13.4±0.20	13.23±0.78	13.12±0.47	13.46±0.52	13.71±0.56	13.29±0.49
C2	13.42±0.42	13.5±0.56	13.10±0.30	13.25±0.61	13.37±0.47	13.44±0.66	13.22±0.48

zetetic actions can be considered as spontaneous pain expressions of the animal [4]. A handheld electronic Von Frey tenderness instrument (North Coast Co. Ltd., USA) was applied to stimulate the trigeminal nerve distribution area. The rat exhibited significant facial scraping and attempted to escape from the stimuli, suggesting that the animal expressed the induced pain. A camera was used to record the behavioural responses in the rat facial regions under different stimulation intensities. The different occurrence accumulated points correspondingly with the highest value being four points. The response behaviour was divided into the following four categories: 1. exploring activities, including sniffing and licking irritants; 2. withdrawing of head from the stimulus; 3. avoiding or aggressive behaviour; 4. grasping and scraping face rapidly and continuously. The scores of the four groups were calculated. Paired t-test was performed for the statistical comparison between the scores of T1 and C1, as well as T2 and C2.

### *Determination of successful modelling*

The experimenter stimulated the rats by touching the cage wall with a handheld stimulating stick for 30 s once. After the rats became accustomed to the stimulation and restored calm, a handheld electronic Von Frey tenderness instrument was used to induce mechanical irritation on the rats. The stimulus intensity was the basic pain threshold. The stimulation site was the vibrissae root section, namely, the section of the infraorbital nerve governed by the facial sensation area. The stimulation was conducted thrice on the operation side, with a time interval of 10 s. The scores recorded in **Table 1** were used to calculate and determine whether the model was successfully established. The spontaneous behavioural and MSNT changes in the four groups were observed ( $P < 0.01$ ).

### *Postoperative behavioural observation*

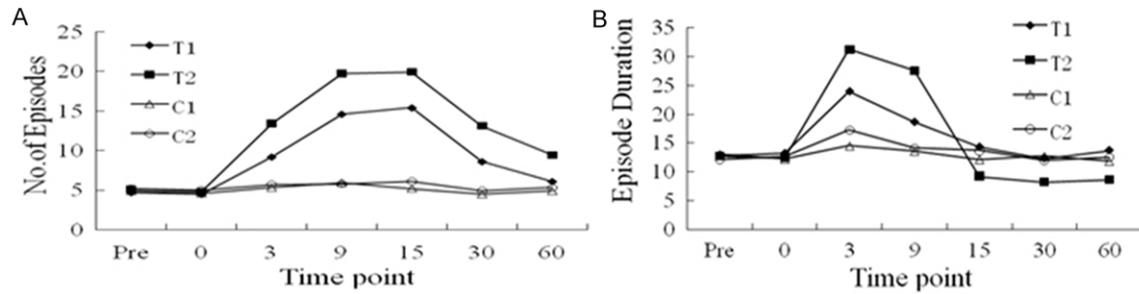
The behaviour changes 3 d before surgery and at six time points (surgery day, postoperative 3, 7, 14, 28, 35 and 42 d) after surgery were investigated. The face of the rat was divided into six regions according to the facial branches of the trigeminal nerve. The results were recorded and divided as 'large, medium and small' [11] using the following description: (1) large: the scraping region was the frontal part; (2) medium: the scraping region was between the infraorbital part and superior part of the lip; (3) small: the scraping region was under the lower lip, submentum and perioral region.

Given that the scraping actions of rats were discontinuous, the scraping segment was defined as a continuous scraping sequence. A time interval was set between two series of scraping (void within the segment), instead of the full terminal (discontinuity between the segments). This criterion was used to determine whether the void was within or between the segments. The list recording method was used to record the duration of each scraping segment [14].

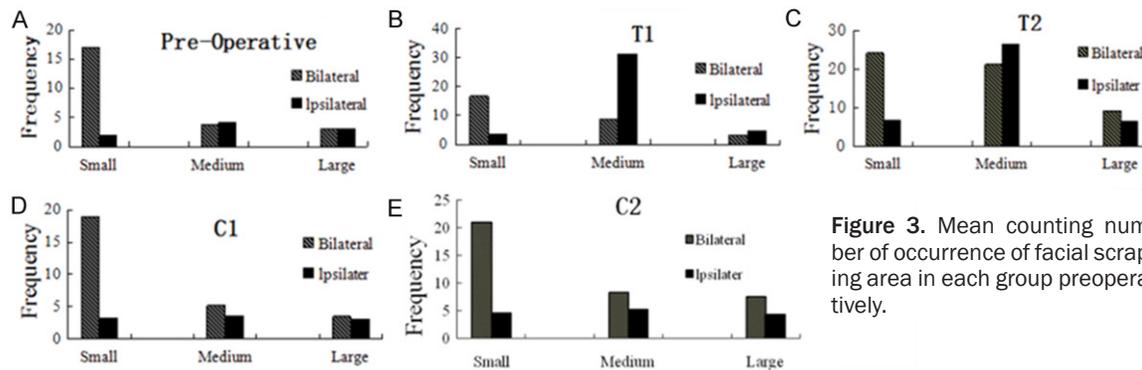
### *Determination of MSNT*

The interference of the external environment was excluded. The rats were individually placed in transparent plastic cages. An experimental stimulus was used to touch the cage wall until the rats adapted to the stimulus. When the rats were in the quiescent state, the real stimulation experiment was implemented. A handheld electronic Von Frey tenderness instrument was used to stimulate the vibrissae root section of the SD rats thrice towards each rat at an interval greater than 10 s. The occurrence of one or more of the following was recorded as the effective stimulus threshold: (1) after the stimulation, escaping actions appeared, such as rapid retreating, turning and curling to avoid irritants, or hiding the head and face under the

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**Figure 2.** The outcomes of the preoperative counting of facial scraping. A: Preoperative counting of facial scraping segments in the 4 groups; B: Preoperative counting of facial scraping segment duration in the 4 groups.



**Figure 3.** Mean counting number of occurrence of facial scraping area in each group preoperatively.

body to protect the face from the irritants; (2) face scratching: appearing as at least three consecutive scrapings of the facial-stimulated region; (3) aggressive behaviour: appearing as rapid grasping and biting the irritants, and making attacking actions. The average value of three test readings was set as the MSNT [14]. Measurements were conducted before surgery and 3, 7, 14, 28, 35 and 42 d after surgery, and the results are recorded in **Table 2**. The preoperatively determined value was set as the basic measurement threshold.

### Statistical analysis

SPSS 13.0 statistical software was used for statistical analysis. Differences between the control and experimental groups were determined using independent samples group t-test. The single-factor ANOVA method was used for T1, T2 and C1, C2, with the statistical significance set at  $P < 0.05$ .

### Results

#### Number of occurrences of facial scraping segments

As shown in **Figure 2A**, no significant difference was observed among the preoperative number

of facial scraping segments in the four groups ( $P > 0.05$ ), and the average number of scraping segments was four to five times. No significant difference was similarly found in the number of facial scraping segments between C1 and C2 groups at any postoperative time point ( $P > 0.05$ ), and the average number of scraping segments was four to six times. By contrast, a significant difference was found in the number of facial scraping segments between C2 and T2 groups ( $P < 0.05$ ) and between T1 and T2 groups ( $P < 0.05$ ).

#### Changes in duration of facial scraping segments

As shown in **Figure 2B**, no significant difference was observed in the preoperative duration of facial scraping segment among the four groups ( $P > 0.05$ ), and the average duration of scraping segments was 13 s to 14 s. No significant difference was also observed in the duration of facial scraping segment between C1 and C2 groups at any postoperative time point ( $P > 0.05$ ), and the average duration of scraping segments was 14 s to 16 s. Before the 15<sup>th</sup> postoperative day, a significant difference was observed in the duration of facial scraping segment between C1 and T1 groups ( $P < 0.05$ ). By

contrast, no significant difference was observed in the duration of facial scraping segment between C1 and T1 groups after the 15<sup>th</sup> postoperative day ( $P > 0.05$ ). At any postoperative time point, a significant difference was found in the duration of facial scraping segment between T2 and C2 groups ( $P < 0.05$ ), as well as between T1 and T2 groups ( $P < 0.05$ ).

### *Frequency of facial scraping regions*

As shown in **Figure 3**, the average number of the preoperative counting of facial scraping regions appeared as the scraping frequency of the bilateral perioral areas (small, bilateral), which exhibited a statistical significance than the scraping frequency in other regions ( $P < 0.05$ ). A statistically significant difference was also observed in the mean scraping number of the infraorbital region than that of the other regions in T1 group (medium, ipsilateral,  $P < 0.05$ ). A statistically significant difference was observed in the mean scraping number of the infraorbital region between T1 and C1 groups ( $P < 0.05$ ). Similarly, a statistically significant difference existed in the mean scraping number of the infraorbital region than that of the other regions in T2 group (medium, ipsilateral; medium bilateral,  $P < 0.05$ ). A statistically significant difference was found in the mean scraping number of the infraorbital region between T2 and C2 groups ( $P < 0.05$ ). By contrast, no statistically significant difference was observed between C1 and C2 groups compared with the preoperative values ( $P > 0.05$ ).

### *Changes in MSNT*

As shown in **Table 2**, the measurements were performed in the four groups before surgery and 3, 7, 14, 28, 35 and 42 d after surgery. The preoperatively determined measurement was set as the basic value of pain threshold.

### **Discussion**

In the 1930s, Dandy [15] described the relationship of cerebellar artery and TNR, and found that it was related to the pathogenesis of TN. In 1967, Jannetta explained the cause of TN using microvascular compression theory, and confirmed his theory through the clinical observation of TN patients. Jannetta [16] found that the onset of original TN is caused by TNR-ectopic vascular compression, and the superi-

or cerebellar artery is the main responsible blood vessel. With the recent development and clinical application of magnetic resonance angiography in scanning the TNR of CPA, the compliance rate of the responsible nerve-root-compressing vessels and clinical confirmation has been found to be more than 93% [17]. The vascular segmental malformations in the CPA, pulsatility and compression can lead to the demyelination changes and short circuits. The tiny tactile stimulation can import the central nervous system through the short circuit, and the transmission impulses generated by the central issue can also be transformed into the incoming impulses through the short circuit, thereby causing intense pain [18, 19].

Studies on neuropathic pain typically use animal models, which have provided effective methods for exploring the developing mechanisms of human neuropathic pain. The preparations of early animal models differed greatly from microvascular nerve root compression theory. Some scholars have injected Freund's adjuvant to stimulate the immune response of TNR and establish the cat dental pulp model [20, 21]. Vos [8] established a neuropathic pain model with chronic constriction ring, and this model is widely used. However, the cerclage tightness on the trigeminal nerve is difficult to control, which results in the difference of the variation degree of nerve fibres and leads to nerve degeneration and necrosis. In addition, the chrome line itself has certain neurotoxicity, which can cause nerve numbness and greater harm to the patient.

Luo [22] established a rat TNR compression model by reaching the TNR through the inferior orbital fissure and inserting a wire to achieve TNR compression. For increased accuracy, Jeon [13] implanted the guiding cannula into the left TNR and injected 4% agar solution with a metal syringe into the dorsal part of left TNR to achieve the compression effect.

In the present study, a cerebral stereotaxic positioning apparatus and nerve plexus stimulator were combined to identify the TNR at the junction of the pons base and middle cerebellar peduncle. When the current stimulated the nerve fibres, the nerve cell membrane underwent depolarisation, generated action potentials and caused a current. Combined with a nerve plexus stimulator, an insulated needle

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was used, which was wrapped with an insulation material, except for the needlepoint so it could have a higher charge density. A small current determined the location of the TNR. This operation was easy with fewer invasions, and could greatly avoid the experimental errors caused by the inaccurate positioning of trigeminal nerve.

The animal models established by the two approaches could generate MSNTs towards the stimulation by a handheld electronic Von Frey tenderness instrument. The ophthalmic and maxillary branches of the trigeminal nerve in SD rat were combined and separated at the orbit tip. The infraorbital tentacles were selected as the main detection area. The two models both generated allodynia towards the mechanical stimulation. The threshold to mechanical stimulation in T1 group began to decrease 7 d later. A significant reduction in stimulation threshold was observed in T2 group 3 d after surgery. The MSNT in T1 group exhibited a rebound phenomenon until about 20 d after surgery. By contrast, the MSNT in T2 group was sustained for 28 d, and gradually exhibited pain threshold rebound 35 d later, which was still significantly higher than that of C2 group. The slight decline of MSNT in C1 and C2 groups within 7 d after surgery was considered the result of surgical stimulation. All experimental SD rats showed weight changes. When animals feel pain, their water and food intake decrease. The severe pain can cause the complete stop of the ingestion of food and water. The reduction of food and water causes the reduction of subcutaneous fat and weight loss. Some rats exhibited loss of skin elasticity, skin that could be easily lifted and dry mucous membranes because of dehydration. In T2 group, the rats exhibited bloody tears at the canthus and large saliva drops at the mouth edge. By contrast, the rats in the other groups did not exhibit these symptoms. This phenomenon was possibly caused by TNR compression by agar. Thus, the traffic branch of the lacrimal secretion-promoting parasympathetic postganglionic fibres of the pterygopalatine ganglion acted with the lacrimal nerve branch of the eye nerve to promote tear secretion. The traffic branch of the parotid secretion-promoting parasympathetic nerve fibres of the ear ganglion reached the parotid with the ear temporal nerve. Furthermore, the submandibular and sublingual secre-

tion-promoting parasympathetic postganglionic fibres of the submandibular ganglion reached the submandibular and sublingual glands, which promoted salivary secretion. This phenomenon confirmed that the TNR agar-compression method was a relatively stable modelling method with strong feasibility.

### Disclosure of conflict of interest

None.

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