

# Stereotaxis of Mandibular Nerve Initial Point of Trigeminal Ganglion in Rats

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**Objective:** To acquire parameters for stereotaxis of the mandibular nerve initial point of the trigeminal ganglion (TG) and to test the accuracy of the acquired parameters for microinjection into the mandibular nerve initial point of TG in adult rats.

**Methods:** Sprague-Dawley rats (260–270 g) were mounted onto a stereotaxic frame. The bregma was set as an anchor point and the three-dimensional parameters between the mandibular nerve initial point of the bilateral TGs and the bregma were measured in 25 rats. The accuracy of these parameters was tested using microinjection of Evans blue dye into the mandibular nerve initial point of the bilateral TGs in 30 rats and the injection sites were evaluated by dissection.

**Results:** The three-dimensional parameters of the mandibular nerve initial point of the bilateral TGs were  $3.5 \pm 0.1$  mm posterior and  $3.6 \pm 0.2$  mm lateral to the bregma, and  $12.0 \pm 0.2$  mm inferior to the skull surface. Accuracy for the microinjection of Evans blue dye into the mandibular nerve initial point of the bilateral TGs was 86.7% (52/60).

**Conclusion:** The acquired parameters served well for stereotaxis and microinjection of reagents into the mandibular nerve initial point of TG.

**Key words:** trigeminal ganglion, stereotaxis, orofacial pain, microinjection, temporomandibular disorders

The trigeminal ganglion (TG), or semilunar ganglion, is a cluster of cell bodies of the trigeminal nerve, which has three major divisions, including the ophthalmic nerve, maxillary nerve and mandibular nerve. By analysing the incoming sensory information, such as pain/temperature and touch/position, from the trigeminal territory, TG provides relay points and intermediary connections between peripheral and central nervous systems<sup>1</sup>. Malfunction of some cytokines, receptors or ion channels in the TG are involved in migraine and orofacial pain, including trigeminal neuralgia, temporoman-

dibular joint (TMJ) inflammatory pain, inflammatory masticatory muscle pain, and tooth pulp inflammatory pain, etc<sup>2-6</sup>. Therefore, TG plays an important role in the pathological process of orofacial pain.

Rats are one of the most commonly used experimental animals and perhaps the one most used in the field of orofacial pain. Intratrigeminal ganglionic injection of reagents such as agonist or antagonist in rats is a feasible and effective method<sup>7,8</sup>. Ahn and colleagues first introduced a reliable rat model of trigeminal neuralgia by injecting 4% agar solution over the surface of the TG to compress the TG<sup>8</sup>. They also made a model of demyelination by injecting lysophosphatidic acid into the TG in rats<sup>7</sup>. However, the accuracy of injection into the TG in rats based on stereotaxis remains obscure. In some cases, it may be necessary to perform precise injections into the mandibular nerve initial point, where the located neurons specifically give off the mandibular nerve of TG to serve different purpose of research, such as TMJ-related pain research.

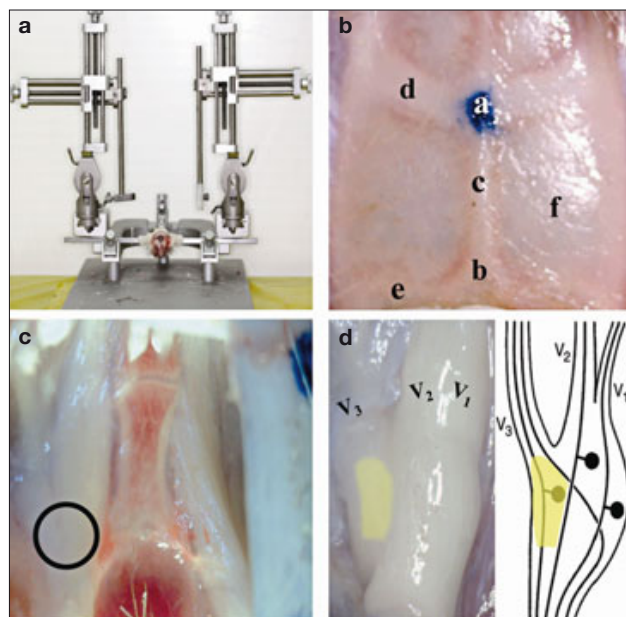
The mandibular nerve innervates a large amount of oral areas, including the skin of the temporal

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**Fig 1** Stereotaxic frame and mandibular nerve initial point of TG. **a** Stereotaxic frame with a rat head in the position. **b** Exposure of anatomical structures over the surface of the parietal bones. a = bregma; b = posterior fontanelle; c = sagittal suture; d = coronal suture; e = interparietal suture; f = parietal bone. **c** Exposure of left TG inside the skull. The black circle indicates the left TG. **d** The mandibular nerve initial point. The left panel is a photograph of a left TG and the right panel is a schematic diagram of the left panel. Yellow shadow: the mandibular nerve initial point (target point), V1 = ophthalmic division, V2 = maxillary division, V3 = mandibular division.

region, auricular, external auditory meatus, cheek, lower lip, lower part of the face, the mucous membrane of the cheek, tongue (anterior two-thirds), mastoid cells, as well as mandibular teeth and periodontal tissues, the mandible bone, parotid, and TMJ. Therefore, intratrigeminal ganglionic injection of agonists or antagonists will be an important method to evaluate the functions of neurons giving off the mandibular nerve. However, there were few studies specifically focusing on whether the injected sites were located in the mandibular nerve initial point of TG. To study the pathological mechanisms of TMJ-related pain or masseter muscle-related pain, it may be necessary to specifically inject reagents into the mandibular nerve initial point, where the located neurons specifically give off the mandibular nerve, of TG in rats. So far, no such data is available yet concerning the stereotaxis of the mandibular nerve initial point of TG in rats.

In this study, we measured the parameters for stereotaxis of the mandibular nerve initial point of TG, and tested the parameters by microinjection of Evans blue dye into the mandibular nerve initial point of TG in rats.

## Materials and methods

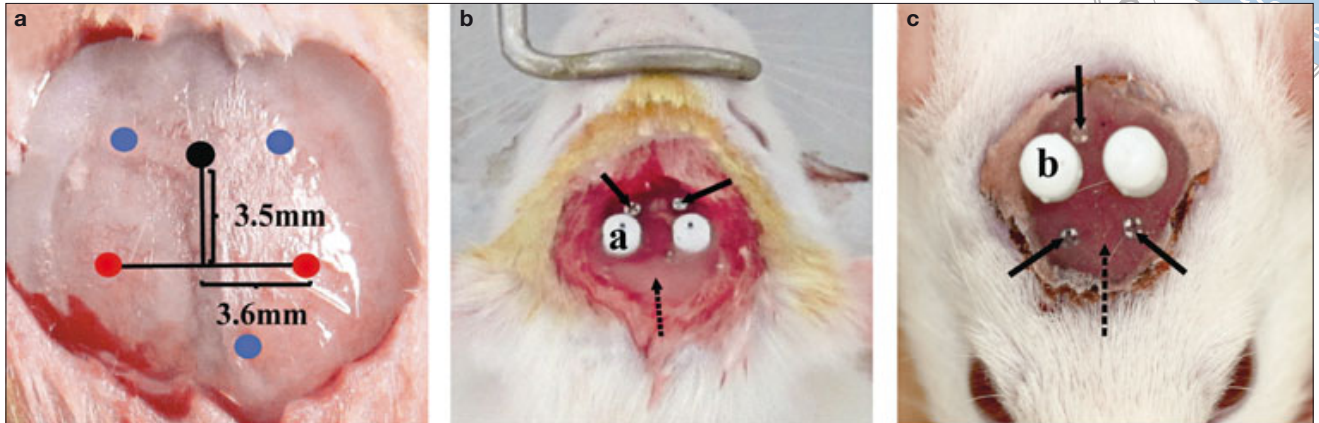
### Animals

Adult male Sprague-Dawley (SD) rats (260–270 g), (purchased from the Academy of Military Medical Sciences, Beijing, China), were housed under a 12 h light/dark cycle in a pathogen-free area with *ad libitum* access to water

and food. The experimental protocols were approved by the Animal Use and Care Committee of Peking University and were consistent with the Ethical Guidelines of the International Association for the Study of Pain.

### Measurement of parameters for stereotaxis of mandibular nerve initial point

Twenty-five rats were killed with an overdose of pentobarbital sodium. The heads of the rats were decapitated and steadily mounted onto a stereotaxic frame (Fig 1a) (model 68001, RWD Life Science Company) through bilateral external auditory canal, keeping the parietal bones parallel with the horizontal plane. The skin over the surface of the parietal bones was excised and the sagittal suture, coronal suture, interparietal suture, bregma, and posterior fontanelle were exposed (Fig 1b). A 10- $\mu$ l Hamilton microsyringe (RWD Life Science Company) was fixed on the holder of the stereotaxic frame, and the tip of the microsyringe was set to touch the bregma in the surface of the skull, which was set as an anchor point. Then the horizontal and vertical coordinates of the tip of the microsyringe were recorded. The bilateral parietal bones were resected, but the sagittal suture region was kept to ensure the position of the rat head was not changed during the resection procedure. The brain tissue was removed to expose the TGs (Fig 1c). By moving the holder horizontally and vertically, the tip of the microsyringe was inserted into the mandibular nerve initial point – where the neurons are located specifically for the mandibular nerve and therefore was the target point (Fig 1d) – until it touched the skull base



**Fig 2** Process of guide cannulae implantation. **a** Locations of key points over the surface of the parietal bones. Black solid dot = bregma; red solid dot = points drilled for cannulae (3.5 mm posterior and 3.6 mm lateral to the bregma); blue solid dot = points drilled for screws. **b, c** Guide cannulae during and after implantation. a = guide cannula; b = stainless steel stylet; solid arrow = stainless steel screws; dotted arrow = dental self-curing acrylic resin.

underneath, and retreated the microsyringe tip upward for 0.5 mm and still leaving the tip within the TG. Then the parameters of the tip of the microsyringe relative to the bregma on the three-dimensional coordinates were recorded and presented as mean  $\pm$  standard deviation (SD). These parameters were used for implantation of the guide cannulae in the test group of rats.

#### Implantation of guide cannulae

The test group consisted of 30 adult male SD rats (260–270 g), which were anaesthetised with pentobarbital sodium (45 mg/kg, ip) and mounted onto the stereotaxic frame. The sagittal suture, coronal suture, interparietal suture, bregma, and posterior fontanelle were surgically exposed as described above. Two guide cannulae (10 mm in working length, 0.56 mm outer diameter and 0.32 mm inner diameter) were implanted into the bilateral TGs according to the parameters acquired above through two small holes drilled in the skull, and then anchored to the skull with dental self-curing acrylic resin, which can provide adequate fixation for the guide cannulae after the implantation, and three stainless steel screws (2 mm in length, 1 mm outer diameter) in triangle distribution were used to reinforce the fixation of guide cannulae connected with dental self-curing acrylic resin to the skull (Figs 2a to 2c). A stainless steel stylet was inserted into the cannula to prevent obstruction and infection (Fig 2c). After the fixation of the guide cannula, local anaesthetic ointment was applied to the skin around the self-curing acrylic resin. Animals were placed on a 37° heating plate for 1 h and maintained under the same

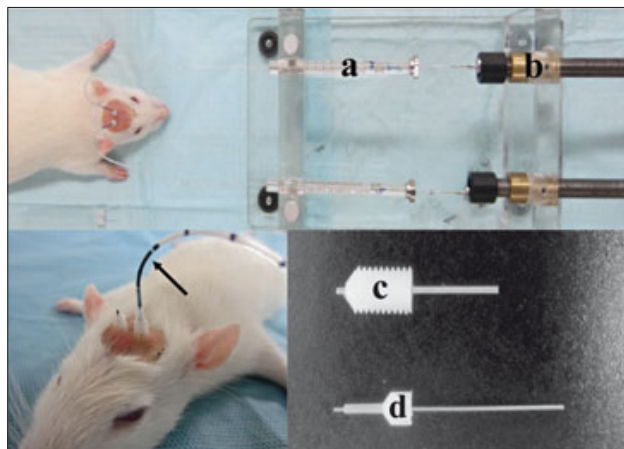
preoperative conditions and fed *ad libitum* for at least 7 days.

#### Behavioural testing

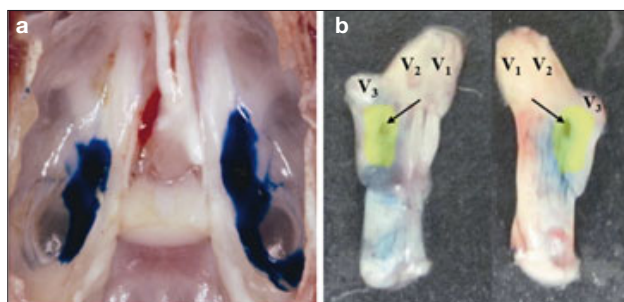
Head withdrawal threshold, which is an indicator of hyperalgesia for the facial region, was measured as reported previously<sup>9-11</sup>. Briefly, head withdrawal threshold was measured before and 7 days after the guide cannulae implantation. The rats were habituated to stand on their hind paws and lean against the experimenter's hand wearing a regular leather working glove. During the whole test session, the rats were unrestrained but remained motionless. The electronic von Frey filament (IITC Life Science) with progressive and increasing forces was applied to the skin of the TMJ region until the head withdrew and meanwhile the applied force was automatically read. Head withdrawal threshold was calculated based on at least five measurements at intervals of 1 min per joint and presented as mean  $\pm$  SD.

#### Confirmation of injection site in TG

After the behavioral testing, the rats were anaesthetised by an overdose of pentobarbital sodium. Microinjections of 2  $\mu$ l of Evans blue dye (Sigma) into the bilateral TGs were performed through a 10- $\mu$ l Hamilton microsyringe fixed on a manual microsyringe pump (68103, Beijing Liandong Yingchuang) and connected by a PE-10 polyethylene catheter with an inner cannula (12 mm in working length, 0.3 mm outer diameter and 0.14 mm inner diameter), which was extended by 2 mm beyond the end



**Fig 3** Instruments for microinjection into TG. a = 10- $\mu$ l Hamilton microsyringe; b = manual microsyringe pump; c = guide cannula; d = inner cannula; solid arrow = Evans blue within a PE-10 polyethylene catheter connecting to the inner cannula.



**Fig 4** Confirmation of injection site in TG. (a) Top view of the bilateral TGs in the original position. (b) Confirmation of the injection sites in TGs after dissection of TGs. Yellow shadow = the mandibular nerve initial point (target point); solid arrow = injection sites located within the region of mandibular nerve initial point. V1 = ophthalmic division; V2 = maxillary division; V3 = mandibular division.

of the guide cannula (Fig 3). The injected sites in the TGs were evaluated by dissection.

#### Statistical analysis

Statistical analysis was performed with SPSS 13 for Windows (SPSS). All data were presented as mean  $\pm$  SD. Differences between groups were examined by one-way analysis of variance (ANOVA), whereas difference between two groups was examined using an independent samples *t*-test. A value of  $P < 0.05$  was considered statistically significant.

## Results

### *Three-dimensional parameters of mandibular nerve initial point relative to bregma*

The three-dimensional distances of mandibular nerve initial point relative to the bregma (the anchor point) were  $3.5 \pm 0.1$  mm posterior and  $3.6 \pm 0.2$  mm lateral to the bregma in the horizontal plane, and  $12.0 \pm 0.2$  mm inferior to the skull surface in the vertical plane.

### *Accuracy of parameters for injection into mandibular nerve initial point*

To test the accuracy of the acquired parameters, we performed a microinjection of Evans blue dye after implanting of the guide cannulae. The injected sites in the TGs were evaluated by dissection. We observed that Evans blue dye was successfully injected into the mandibular nerve initial point of the bilateral TGs in 26 (52 TGs) out of 30 rats (60 TGs) (52/60, 86.7%) (Fig 4), indicating that the parameters served well for stereotaxis of the mandibular nerve initial point of TG in adult rats.

### *Algesia behaviors were not affected after implantation of guide cannulae*

As shown in Fig 5, head withdrawal threshold was not different on day 7 post-implantation of the guide cannulae from that of pre-implantation.

## Discussion

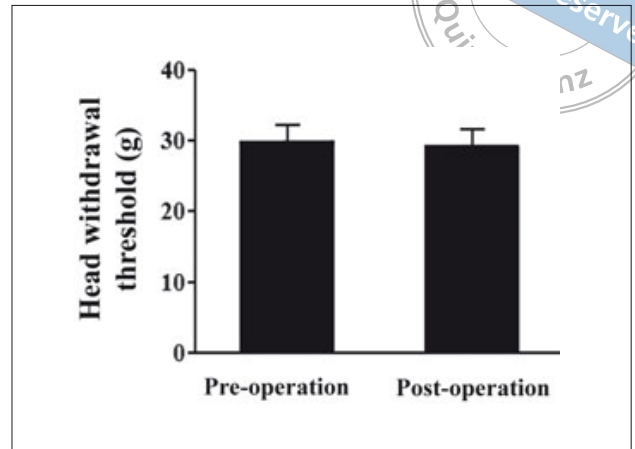
The present study measured the parameters for stereotaxis of the mandibular nerve initial point of TG and tested the accuracy of the parameters by implantation of the cannulae and microinjection of Evans blue dye into it. We found that the acquired parameters served well for the microinjection into the mandibular nerve initial point of TG at an accurate rate of 86.7%, and that the implantation of the cannulae into the mandibular nerve initial point did not affect the responses of the rats to the mechanical stimuli. Neurons located in the mandibular nerve initial point give off the mandibular nerve providing sensory innervation to the TMJ, mandibular teeth, the skin and mucous membrane of cheek, and the fascia of masticatory muscles, etc. Therefore, the parameters we acquired were specifically for the stereotaxis of the mandibular nerve initial point and would help studies which are interested in analysing the functional changes of the region innervated by the mandibular nerve by injecting reagents such as agonists or antagonists into

the mandibular nerve initial point of TG. It may be especially useful for studying the potential pathological mechanisms of TMJ or masseter muscle inflammatory pain.

Microinjection of agonist or antagonist into the mandibular nerve initial point of TG in rats has an important significance to directly observe the effects of the corresponding ion channels or receptors in the TG related to TMJ pain or masseter muscle pain, since rats are preferred and usually used in the field of orofacial pain. However, blocking the functions of the neurons specifically for the mandibular nerve depends on accurate injection of the reagents into the mandibular nerve initial point of the TG. In the present study, we set the bregma as an anchor point as described previously<sup>8</sup>, and obtained the three-dimensional parameters relative to the bregma for stereotaxis of the mandibular nerve initial point and tested the parameters through the microinjection of Evans blue dye into the mandibular nerve initial point in 30 rats, and showed the parameters were reliable.

However, there were still eight injections that missed the mandibular nerve initial point (4 out of 30 rats), suggesting some other factors might influence the accuracy of stereotaxis of or microinjection into the mandibular nerve initial point of the TG of rats. We reviewed the procedures of implantation of the guide cannulae and microinjection, and believed that attention might be paid to the following steps. First, after mounting the rat head onto the stereotaxic frame and adjusting the parietal bones parallel with the horizontal plane, the rat head should be completely immobilised during the implantation of the guide cannula, because subtle movement of the rat head during the implantation would notably change the bregma's three-dimensional positions. Second, the guide cannula should be vertically fixed onto the skull and not separate the guide cannula from the holder until the self-curing acrylic resin totally solidified, because even a slight movement in any direction of the top of the cannula outside the skull could cause a big movement of the end of the cannula inside the skull. Third, the working length of inner cannula should be long enough (12 mm) to keep its tip within the TG, whereas the guide cannula should keep its tip just touching onto the surface of the TG (10 mm) to prevent any possible compression on it.

Implantation of the guide cannula into the TG did not affect mechanical allodynia behaviours of rats. The process of insertion of the guide cannula into the TG would cause the destruction of some portions of the brain, including parts of the cortex and thalamus, and might affect allodynia behaviours. However, we observed



**Fig 5** Head withdrawal thresholds before and after implantation of guide cannulae. Head withdrawal threshold was not changed on day 7 after implantation of the guide cannulae. ( $P > 0.05$  versus pre-operation,  $n = 30$ ).

that after implanting the guide cannula into the mandibular nerve initial point, the head withdrawal threshold of the rats did not show any difference from that of the pre-implantation, suggesting that implantation of the cannula might not affect rat mechanical allodynia behaviour. Also, no abnormality of locomotor system of rats were observed after the guide cannula implantation. Therefore, our results of mechanical allodynia behaviour in rats supported the observation that the function of a certain ion channel or receptor in the TG could be achieved through microinjection of its agonist or antagonist into the TG.

In summary, we acquired the three-dimensional parameters for stereotaxis of the mandibular nerve initial point of rat TG, and tested their accuracy through microinjection of Evans blue dye into the TG. Our results showed the acquired parameters served well for stereotaxis of or microinjection of reagents into the mandibular nerve initial point, which would be useful for orofacial pain related research.

## References

1. Purves, D Augustine GJ, Fitzpatrick D, et al. Neuroscience, ed 2. Sunderland: Sinauer Associates. 2007.
2. Nordgaard JC, Kruse LS, Møller M, Kruse C. Phosphodiesterases 3 and 5 express activity in the trigeminal ganglion and co-localize with calcitonin gene-related peptide. Cephalalgia 2013;34:503–513.
3. Chen Y, Williams SH, McNulty AL, et al. Temporomandibular joint pain: a critical role for Trpv4 in the trigeminal ganglion. Pain 2013;154:1295–1304.

4. Bai G, Ambalavanar R, Wei D, Dessem D. Downregulation of selective microRNAs in trigeminal ganglion neurons following inflammatory muscle pain. *Mol Pain* 2007;3:15.
5. Sabalys G, Juodzbaly G, Wang HL. Aetiology and Pathogenesis of Trigeminal Neuralgia: a comprehensive review. *J Oral Maxillofac Res* 2013;3:e2.
6. Tarsa L, Bałkowiec-Iskra E, Kratochvil FJ 3rd, et al. Tooth pulp inflammation increases brain-derived neurotrophic factor expression in rodent trigeminal ganglion neurons. *Neuroscience* 2010;167:1205–1215.
7. Ahn DK, Lee SY, Han SR, et al. Intratrigeminal ganglionic injection of LPA causes neuropathic pain-like behavior and demyelination in rats. *Pain* 2009;146:114–120.
8. Ahn DK, Lim EJ, Kim BC, et al. Compression of the trigeminal ganglion produces prolonged nociceptive behavior in rats. *Eur J Pain* 2009;13:568–575.
9. Ren K. An improved method for assessing mechanical allodynia in the rat. *Physiol Behav* 1999;67:711–716.
10. Kou XX, Wu YW, Ding Y et al. 17beta-estradiol aggravates temporomandibular joint inflammation through the NF-kappaB pathway in ovariectomized rats. *Arthritis Rheum* 2011;63:1888–1897.
11. Wu YW, Bi YP, Kou XX, et al. 17-Beta-estradiol enhanced allodynia of inflammatory temporomandibular joint through upregulation of hippocampal TRPV1 in ovariectomized rats. *J Neurosci* 2010;30:8710–8719.

