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Original Reports

An Improved Rodent Model of Trigeminal Neuropathic Pain by Unilateral Chronic Constriction Injury of Distal Infraorbital Nerve

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Abstract: **The number of studies on trigeminal nerve injury using animal models remains limited. A rodent model of trigeminal neuropathic pain was first developed in 1994, in which chronic constriction injury (CCI) is induced by ligation of the infraorbital nerve (IoN). This animal model has served as a major tool to study trigeminal neuropathic pain. Unfortunately, the surgical procedure in this model is complicated and far more difficult than ligation of peripheral nerves (eg, sciatic nerve). The aim of this study was to improve on the current surgical procedure of IoN ligation to induce trigeminal neuropathic pain in rats. We show that the IoN can be readily accessed through a small facial incision. CCI can be induced by ligation of a segment at the distal IoN (dIoN). This dIoN-CCI procedure is simple, minimally invasive, and time-saving. Our data show that the dIoN-CCI procedure consistently induced acute as well as chronic nociceptive behaviors in rats. Daily gabapentin treatment attenuated mechanical allodynia and reduced face-grooming episodes in dIoN-CCI rats.**

Perspective: ***The orofacial pain caused by trigeminal nerve damage is severe and perhaps more debilitating than other types of neuropathic pain. However, studies on trigeminal neuropathic pain remain limited. This is largely because of the lack of proper animal models because of the complexity of the existing surgical procedures required to induce trigeminal nerve injury. Our improved dIoN-CCI model is likely to make it more accessible to study the cellular and molecular mechanisms of neuropathic pain caused by trigeminal nerve damage.***

Key words: *Rat, distal, infraorbital nerve, orbital cavity, chronic constriction, mechanical allodynia, face-grooming.*

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Orofacial neuropathic pain caused by trigeminal nerve injury is a debilitating condition with limited therapeutic options.[11,27] Chronic constriction injury (CCI) of the infraorbital nerve (IoN) was developed in 1994[25] and has been used as a major animal model to study trigeminal neuropathic pain disorders. IoN-CCI induces injury to a pure sensory nerve resulting in painful sensory disturbances in the IoN territory such as the mystacial vibrissae and its surrounding hairy skin areas.[7,23] Thus, IoN-CCI appears to be a suitable animal model to study neuropathic pain caused by trigeminal nerve injury.

However, trigeminal neuropathic pain disorders are not as widely studied as peripheral neuropathic pain conditions probably because of the complexity of the IoN-CCI

procedure.[25] In this study we demonstrate a simplified, minimally invasive, and time-saving surgical procedure to produce CCI by the ligation of a distal IoN (dIoN). We compared and contrasted our procedure with the procedure originally developed by Vos et al,[25] in which an intraorbital segment of the IoN was ligated. We also made comparison with a procedure in which the ligation site on the IoN is distal to the infraorbital foramen.[10] Our data show that the dIoN-CCI procedure consistently induced acute as well as chronic nociceptive behaviors in rats that were comparable with those induced by more complex and more traumatic surgical procedures.

Methods

Animals

Adult male Sprague Dawley rats weighing 250 to 270 g were purchased from Charles River Laboratories (Wilmington, MA). The experimental protocols were approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee.

Surgical Procedures

All surgical procedures were performed aseptically on rats anesthetized with ketamine/xylazine (75 mg/kg; 5 mg/kg). The shaved skin surface was scrubbed with iodine, and 70% isopropyl alcohol was used to remove excess iodine. We used different surgical procedures in this study, as listed in the following sections, to compare the behavioral outcome reflective of trigeminal neuropathic pain.

dIoN-CCI, Ligation of a Distal Segment of the IoN Outside the Orbital Cavity Through a Facial Incision

The facial surface between the eye and whisker pad of the rat was gently shaved without damaging the whiskers. A .5 cm incision parallel to the midline was made starting at the caudal end of the third row of whisker lines toward the ipsilateral orbit (Fig 1A). The superficial fascia was bluntly separated to expose the IoN trunk at its distal segment outside the orbital cavity (Fig 1B). Two chromic catgut ligatures (4-0) were loosely tied around the distal part of the IoN (2 mm apart; Figs 1C and D). To ensure proper constriction of the IoN, a criterion proposed by Bennett and Xie[2] was followed. The ligatures were applied in such a way that the diameter of the IoN was reduced by a just noticeable amount and the circulation through the superficial vasculature was retarded but not cut off. The skin incision was closed with a polyester suture (4-0; Fig 1E). Rats in sham groups underwent the same surgical procedure including skin incision and the IoN nerve dissection except for the actual nerve ligation.

IoN-CCI, Ligation of an IoN Segment Inside the Orbital Cavity Through a Midline Incision

The unilateral ligation of the IoN was performed as described by Vos et al.[25] We shaved the head of the rat and fixed the head in a stereotaxic frame. We made a midline incision on the scalp to expose skull and nasal bone (Fig 1F). The boundaries of the orbit were exposed by tissue dissection, including the maxillary, frontal, lacrimal, and zygomatic bones. We exposed the IoN by gently deflecting the orbital contents with a cotton swab. The IoN was then dissected from its surrounding tissues at its most rostral extent in the orbital cavity, which was just caudal to the infraorbital foramen (Fig 1G). Two chromic catgut ligatures (4-0) were loosely tied around the IoN (2 mm apart; Figs 1H and I). The skin incision was closed with 3 polyester sutures (4-0; Fig 1J).

IoN-CCI, Ligation of an IoN Segment Outside of the Orbital Cavity Through a Midline Incision

A midline incision was made over the nose of the rat as described[10] (Fig 1K). The left IoN was exposed just distal to the IoN foramen[10] (Fig 1L). Two 4-0 chromic gut sutures were placed around the IoN just distal to the foramen[10] (Figs 1M and N). Each suture was loosely placed around the nerve. The skin incision was closed with 3 polyester sutures (4-0; Fig 1O).

Neuronal Tracing

Anterograde and retrograde labeling tracer 3000 Da tetramethylrhodamine-conjugated dextran (Invitrogen, Carlsbad, CA) was used to label the neurons in the trigeminal ganglion.[15] The IoN trunk at its distal segment outside the orbital cavity was exposed (Fig 1B). The IoN trunk was transected at the same site as for ligation. To prevent tracer from contaminating the surrounding tissue, a small piece of parafilm was placed under the transected IoN. Dimethyl sulfoxide was applied to the transected surface of the IoN to increase the penetration of dextran. Dextran granules were applied directly onto the transected surface of the IoN with a microspatula and held in place for 1 minute. A small dab of petroleum jelly was applied to the nerve end before sealing it with parafilm and superglue. At 2, 5, and 7 days after dextran application, rats were perfused with 4% of paraformaldehyde as described previously.[29] Brains and the trigeminal ganglion were harvested, sectioned, mounted on the glass slides, and examined using an Olympus fluorescence microscope (Olympus Corp, Tokyo, Japan).[29] The site of facial nucleus in the brain section was determined on the basis of stereotaxic coordinates of The Rat Brain.[8]

Drug Treatment

Gabapentin was administered daily using oral gavage using an autoclavable curved rat feeding tube with a ball tip (Kent Scientific, Torrington, CT). The feeding volume was determined on the basis of body weight at .1 to .2 mL/10 g.[4] The dose of gabapentin was 150 mg/kg.[5]

Behavioral Tests

All behavioral experiments were carried out with the investigators blinded to treatment conditions.