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Research report

Assessment of chronic trigeminal neuropathic pain by the orofacial operant test in rats

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HIGHLIGHTS

- The orofacial operant test was used to assess chronic pain in rats with infraorbital nerve injury.
- Operant behaviors revealed mechanical and cold allodynia and cold hyperalgesia in these rats.
- Operant behaviors also revealed pain relief by morphine in these trigeminal pain animals.
- Orofacial operant test is a desirable method for studying chronic trigeminal neuropathic pain.

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ABSTRACT

Classical behavioral tests in animal models of trigeminal neuropathic pain measure reflexive responses that are not necessarily measures of pain. To overcome the problem, we created a chronic constrictive nerve injury (CCI) rat model of pain by ligation of the infraorbital nerve (ION), and applied the orofacial operant test to assess behavioral responses to mechanical and cold stimulation in these rats. Animals were trained to voluntarily contact their facial region to a mechanical or a cold stimulation module in order to access sweetened milk as a positive reward. ION-CCI rats displayed aversive behaviors to innocuous mechanical stimuli, as indicated by a significant decrease in both contact time and the numbers of long contact events in comparison with sham group. For cold stimulation, ION-CCI rats displayed aversive behaviors to both innocuous (17 °C) and noxious cold temperatures (12 °C and 5 °C), as indicated by a significant decrease in both contact time and the numbers of long contact events at the cooling temperatures. The decreases of the contact time and numbers in ION-CCI rats were partially abolished by morphine. Our orofacial operant test demonstrates mechanical allodynia, cold allodynia, and hyperalgesia in rats with chronic trigeminal nerve injury. The neuropathic pain in ION-CCI rats was partially alleviated by morphine. Thus, orofacial operant test provides a desirable behavioral assessment method for preclinical studies of chronic trigeminal neuropathic pain.

1. Introduction

Trigeminal neuralgia is the most common debilitating orofacial neuropathic pain disorder [1,2]. It is often manifested with thermal and mechanical allodynia and hyperalgesia [2,3]. For example, trigeminal neuralgia patients can suffer severe pain triggered by a gentle air puff on their faces. This neuropathic pain disorder, among various others (e.g. temporomandibular disorders), can be difficult to treat since the pathophysiology is not well understood [2]. To better study neuropathic pain, Bennett and Xie [4] first developed the chronic constriction injury (CCI) model in rats. This method consists of tying ligatures around a sciatic nerve trunk to produce neuropathic pain states including thermal and mechanical allodynia and hyperalgesia in rat hindpaws. Orofacial regions are innervated by the trigeminal nerve system, the cranial sensory system that shares many similarities to the sciatic nerves. However, the trigeminal system has features different from the sciatic nerves. For example, trigeminal nerve branches that innervate dental pulps of the teeth are exclusively nociceptors [5]. The study of trigeminal neuropathic pain has been facilitated by adopting the sciatic CCI method to the infraorbital nerve (ION) [6,7]. The ION, the entire second division of the trigeminal nerve, is exclusively sensory and covers the most common distribution for trigeminal neuralgia in orofacial region.

In most previous studies, classical behavioral tests such as von Frey filament poking for mechanical stimulation were applied to orofacial regions [6–8]. ION-CCI animals have demonstrated behavioral alterations including paw licking, face-rubbing,

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limb-guarding, vocalization, grooming [6,9]. However, these are unlearned behaviors that are reflexive responses mediated by brainstem since these behaviors could be seen in decerebrate animals [10]. Although useful in preclinical studies, these reflex behaviors do not provide information on a higher order cerebral function and are not necessarily measures of pain [11]. There are also technical concerns on the previous orofacial behavioral tests, including stress of animals in restrained condition, anticipation of stimulation as animals can visualize probes approaching them, and investigator bias. These problems may account for the large variations in previous orofacial pain behavioral tests.

In view of the problems of classical behavioral tests, Neubert et al. [12] has developed an orofacial operant test system. Operant tests of pain use a conflict paradigm to allow animals to make a choice between receiving a positive reward (drinking sweetened milk) or escaping aversive stimuli [12], and animals have control over the amount of nociceptive stimulation and can modify their behavior based on cerebral cortical processing [13,14]. Therefore, operant behavioral responses are not simple reflexive responses and are considered to be better indicator of pain in comparison with classical behavioral tests. Orofacial operant tests have been used to characterize thermal pain in normal rats following noxious heat or cold stimulation [15,16], thermal and mechanical pain in rats with facial inflammation induced by injection of carrageenan and capsaicin [12,17]. To the best of our knowledge, there is no reported study applying orofacial operant behavior paradigm to study trigeminal neuropathic pain.

2. Materials and methods

Male Sprague-Dawley rats (280–380 g) were used in this study. All animals were exposed to light 12 h per day; food and water were available ad libitum. Protocol for the maintenance and use of the experimental animals were approved by the Laboratory Animal Medical Services and Institutional Animal Care and Use Committee at the University of Cincinnati. These were carried out in accordance with the NIH regulations on animal use.

Animals initially underwent 2 weeks of pre-surgical adaptation training utilizing the Ugo Basile Orofacial Stimulation Test System® (Comerio VA, Italy) after a 12 h fasting food period. Rats were placed in a standard rat cage with a plastic divider to create two rooms, the testing room and the companion room. In the anterior aspect of the cage there was an Ugo Basile apparatus with a drinking window for the rat head to enter and acquire a reward (milk) located on the opposing aspect of the drinking window. Nestle Carnation® sweetened condensed milk was diluted with deionized water to 30% and placed in a cylindrical plastic container with metal nipple drinker. The apparatus also consisted of a mechanical or a thermal module but the module was removed during adaptation training period. An infrared photo-beam was built on the exterior aspect of the drinking window and wired to a computer to automatically detect head accessing the feeding tube. Depending on the type of the experiment, the animals were subjected to either no stimulus during the adaptation training period, mechanical, or thermal stimulus when it attempted to poke its head through the drinking window. For the adaptation training, the orofacial apparatus was used without stimulation modules. The training was started by placing a rat in the testing room and another one in the companion room. After the rats were given 10 min to familiarize themselves with their environment, the drinking window was opened and the testing rat was subsequently timed for 10 min to allow drinking the milk.

After 2 weeks of the pre-surgical adaptation training, the rats were divided into two groups: sham and ION-CCI (infraorbital nerve ligation). In the ION-CCI group a chronic constriction nerve injury model was created using unilateral ligation of the infraorbital nerve as described previously [7] In brief, the rats were anesthetized with intraperitoneal injection of ketamine/xylazine cocktail (100 mg/kg). The skin above the right eye was shaved and the rat head was immobilized. A 2-cm curvilinear incision was made superior to the right orbital cavity. A meticulous dissection was made, and the muscle and fascia was retracted laterally. The infraorbital nerve can be found approximately 1 cm down against the floor of the maxillary bone. The nerve was freed from the surrounding connective tissues and two ligatures were made approximately 5 mm apart with a 5-0 absorbable chromic gut suture Superion®. The incision was closed with 6-0 non-absorbable braided silk suture. The sham groups also had a similar surgery, but without any ligatures. The nerve was freed from the surrounding connective tissue and the incision closed. After a 2-weeks healing period, the rats underwent a 2-weeks period of post-surgical adaption training performed in the same manner as the pre-surgical adaptation training.

Subsequently, experiments were performed utilizing the mechanical module or thermal module during post-operative period of 4–8 weeks. The mechanical module was custom made. It consisted of a cassette with ten tungsten wires placed 3 mm apart from each other and at 8 mm from the opening hole to the cassette held to produce a bending force from the drinking window of the apparatus. The proximal tips of these wires were coated with a drop of ethyl 2-cranaocrylate to create blunt tips. For mechanical stimulation experiments, the animal's face contacted the tungsten wires of mechanical module as it projected its head through the hole in the apparatus in order to drink milk located on the exterior aspect of the drinking window. In the thermal module there was a surrounding metal tubing at the opening enclosed with circulating ethylene glycol (Sigma–Aldrich, US) made in a 50/50 mixture with distilled water. The temperatures of the circulating ethylene glycol solution were controlled by a thermal circulating bath unit. The distance between the metal tube and the nipple of the milk bottle was 14 mm. For thermal stimulation, thermal module was set at 24 °C, 17 °C, 12 °C, or 5 °C. The animal's orofacial region was shaved and subjected to different cold stimuli by contacting the metal tube as it poked its head through the hole to obtain the milk. To test the effects of morphine on mechanical and cold sensitivity of ION-CCI rats, animals were administered morphine (s.c., 0.5 mg/kg) 30 min prior to the orofacial operant tests. At different days these animals were also administered saline as control and then orofacial operant tests were performed in the same manner. Similar to the adaptation training, all experiments with mechanical or thermal module were preceded by a 12 h fasting period, 10 min for the rats to be familiarized with testing environment, and a subsequent 10 min to allow for orofacial operant behavioral assessment.

The events of head pokes were detected by the infrared photo-beam, recorded by a computer, and analyzed by the Oro software (Ugo Basile, Comerio VA, Italy). This computer software recorded and analyzed several variables of the rat's behavior including the total time the beam was broken also defined as the contact time, the count, which can also be described as number of contacts, and the maximum as well as the minimum contact time. All data were analyzed by the one way ANOVA test. Post hoc comparisons were made using the Duncan's test. Significant value for this statistical method was at $P < 0.05$.

3. Results

3.1. Experimental settings and general observations

We modified the Ugo Basile Orofacial Stimulation Test System® to create a companionship environment (Fig. 1A) that would expedite the adaptation of the testing rat and promote a consistent drinking behavior. When stimulation modules were placed in the drinking window, drinking behaviors were found to be also affected by the positions of the nipple of milk bottle and infrared beam. The locations of these parts were tested in preliminary studies and the optimal distances were maintained (Fig. 1B) in each set of experiments. A custom made mechanical stimulation module was used for the mechanical stimulation (Fig. 1C left). The module consisted of a cassette that anchored tungsten wire filaments. The length of each filament was set to 8 mm to produce desirable bending forces (Fig. 1D). Our preliminary study, performed at 2 mm intervals, confirmed that the filament length of 8 mm created the optimum behavior results that could well differentiate ION-CCI group from sham group (Fig. 1E). At filament length of 6 mm, all rats had excessive restriction and at 10 mm they would drink the milk with comfort such that differences between sham and ION-CCI groups were not as significant as those at 8 mm. Once this optimal length of filament was established, experiments were carried out at several access distances, defined as the distance between the mechanical module to the metal nipple drinker, to determine the optimum access distance at which the mechanical stimulation altered orofacial operant behavior of the ION-CCI rats but least affected the sham rats. The second part of the study was to test the outcome of innocuous and noxious cold allodynia on orofacial operant behaviors by using a thermal stimulation module (Fig. 1C right). In order to create an orofacial region that is more sensitive to cooling temperatures, the sham and ION-CCI group rat's facial area were shaved (Fig. 2A top panel) one day before experimentation. The orofacial region is innervated by infraorbital nerve from V2 branch of trigeminal nerve (Fig. 2A middle panel). The ligation of infraorbital nerve (Fig. 2A middle panel) would produce nerve