Neuroscience 295 (2015) 126–138

TRIGEMINAL INFLAMMATORY COMPRESSION (TIC) INJURY INDUCES CHRONIC FACIAL PAIN AND SUSCEPTIBILITY TO ANXIETY-RELATED BEHAVIORS

D. N. LYONS,[a] T. C. KNIFFIN,[b] L. P. ZHANG,[a] R. J. DANAHER,[c] C. S. MILLER,[c] J. L. BOCANEGRA,[c] C. R. CARLSON[b] AND K. N. WESTLUND[a]*

[a] *Department of Physiology, University of Kentucky, United States*

[b] *Department of Psychology, University of Kentucky, United States*

[c] *Departmentof Oral Health Practice, University of Kentucky, United States*

Abstract—Our laboratory previously developed a novel neuropathic and inflammatory facial pain model for mice referred to as the Trigeminal Inflammatory Compression (TIC) model. Rather than inducing whole nerve ischemia and neuronal loss, this injury induces only slight peripheral nerve demyelination triggering long-term mechanical allodynia and cold hypersensitivity on the ipsilateral whisker pad. The aim of the present study is to further characterize the phenotype of the TIC injury model using specific behavioral assays (i.e. light–dark box, open field exploratory activity, and elevated plus maze) to explore pain- and anxiety-like behaviors associated with this model. Our findings determined that the TIC injury produces hypersensitivity 100% of the time after surgery that persists at least 21 weeks post injury (until the animals are euthanized). Three receptive field sensitivity pattern variations in mice with TIC injury are specified. Animals with TIC injury begin displaying anxiety-like behavior in the light–dark box preference and open field exploratory tests at week eight post injury as compared to sham and naïve animals. Panic anxiety-like behavior was shown in the elevated plus maze in mice with TIC injury if the test was preceded with acoustic startle. Thus, in addition to mechanical and cold hypersensitivity, the present study identified significant anxiety-like behaviors in mice with TIC injury resembling the clinical symptomatology and psychosocial impairments of patients with chronic facial pain. Overall, the TIC injury model's chronicity, reproducibility, and reliability in producing pain- and anxiety-like behaviors demonstrate its usefulness as a chronic neuropathic facial pain model.

Key words: chronic orofacial pain, stress-induced analgesia, acoustic startle, mouse model, operant tests, nerve injury.

INTRODUCTION

Approximately 22% of the US population suffers from facial and headache pain. Patients with trigeminal neuropathic pain, one type of chronic facial pain, frequently report experiencing a continuous aching and burning pain sensation that may be accompanied by intermittent electrical shock-like pain. Patients with this type of facial pain also report mechanical allodynia and cold hypersensitivity (Baron et al., 2010; Zakrzewska, 2013a,b). While dental procedures or trauma are known causes of peripheral trigeminal nerve injury and inflammation, in some cases, no clear causes are identified for the origin and maintenance of trigeminal neuropathic pain (Porto et al., 2011; Renton and Yilmaz, 2011).

There are, however, a limited number of models of such pain conditions available for use in laboratory experiments. Historically, one model of neuropathic, facial pain frequently used in rats is known as the chronic constriction injury of the infraorbital nerve (CCI-ION) (Vos and Strassman, 1994). This model has been adapted for use in mice and is referred to as the partial CCI-ION (Xu et al., 2008). Both models involve tying chromic gut suture around the ION, a branch of the maxillary nerve which innervates the whisker pad of rodents, which causes mechanical hypersensitivity in the whisker pad region. However, tying this suture causes deformation of the ION and constricts blood flow thus inducing partial nerve ischemia and loss (Bennett and Xie, 1988; Kim and Chung, 1992; Kawamura et al., 1997), features not consistent with the clinical symptomatology of patients suffering from trigeminal neuropathic pain. To address these issues, a novel chronic facial neuropathic pain model in mice, named the Trigeminal Inflammatory Compression (TIC) injury model, was developed in our laboratory to more closely mimic the clinical characteristics of trigeminal neuropathic pain (Ma et al., 2012). As previously reported, the TIC injury model is produced by inserting chromic gut suture between the infraorbital nerve and the maxillary bone. This placement alongside the nerve, rather than constriction of the nerve, has been successful in limiting whole nerve ischemia and demyelination in mice but promoting long-term whisker pad hypersensitivity (Ma et al., 2012).

The present studies were performed by another surgeon and other testers than in the original study to validate the method. Due to its novelty, reliability, and relevance for translational studies, there is a great need

*Corresponding author. Address: Department of Physiology, MS-508 College of Medicine, University of Kentucky, Lexington, KY 40536-0298, United States. Tel: +1-859-323-3668; fax: +1-859-323-1070.
E-mail address: kwhigh2@uky.edu (K. N. Westlund).

Abbreviations: CCI-ION, chronic constriction injury of the infraorbital nerve; TIC, Trigeminal Inflammatory Compression.

http://dx.doi.org/10.1016/j.neuroscience.2015.03.051
0306-4522/

for further characterization of the TIC injury model to increase our understanding of the behavioral characteristics of the model. For example, one important aspect of the clinical presentation of trigeminal neuropathic pain is the common comorbidity of psychological disorders and emotional distress (Wall and Melzack, 1999). In clinical populations, symptoms of anxiety and depression in particular have been consistently observed in patients with chronic trigeminal-mediated pain (Averill et al., 1996; Fishbain, 1999a, b, McWilliams et al., 2003; Nicholson and Verma, 2004; Robinson et al., 2009; Burris et al., 2010).

The measurement of constructs such as anxiety and depression in animal models, however, has proven more difficult than in clinical populations. Fortunately, the use of cognitive-dependent tests offers a more thorough examination of psychological constructs such as anxiety, and are increasingly used by researchers seeking to understand chronic neuropathic pain conditions (Mao et al., 2008; Mogil, 2009). Measures of anxiety-like behaviors in animals have been extensively studied, and numerous validated protocols have been developed (Belzung and Griebel, 2001). Three assays that are particularly well understood in measuring animal behavior associated with psychological constructs such as anxiety are: the light–dark preference test, the open field exploratory test, and the elevated plus maze task. Furthermore, the activity and rearing behavior in each of these tasks has been previously shown to be affected by pain (Crawley and Goodwin, 1980; Belzung and Griebel, 2001; Bouwknecht and Paylor, 2002; Roeska et al., 2008; Parent et al., 2012).

The aim of the current study was to further characterize the novel TIC injury model by examining mechanical allodynia and heat hypersensitivity, as well as by measuring anxiety-like behaviors with cognitive-dependent operant tests. The hypothesis was that mice with TIC injury would display greater mechanical allodynia, cold hypersensitivity, and more anxiety-like behaviors than naïve mice or animals undergoing sham surgical procedures.

EXPERIMENTAL PROCEDURES

Animals

All experiments were performed with C57Bl/6 male, wild-type mice that weighed between 25 and 35 g purchased from the Harlan Laboratories (Indianapolis, IN, USA). All mice were age matched. Animals were randomly assigned to receive either experimental (TIC injury model) surgical procedures, sham surgical procedures, or to remain naïve. Mice were housed in a well-ventilated room (maintained at 27 °C) with a reversed 10/14-h dark/light cycle so that testing could be performed during the active period. All mice had access to food and water *ad libitum* throughout the duration of the experiment. Regular rodent chow diet with low soy bean content was provided (Teklab 8626, Harlan, IN, USA). All experimental procedures were completed according to the guidelines provided by the National Institute of Health (NIH) regarding the care and use of animals for experimental procedures. Animal protocols were approved by the University of Kentucky's Institutional Animal Care and Use Committee (IACUC). All animals were housed in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and the United States Department of Agriculture (USDA).

TIC injury surgery

Mice were anesthetized with sodium pentobarbital (70 mg/kg, i.p.). Under standard sterile surgery conditions, the hair on the top of their head was shaved and the area disinfected. Ophthalmic cream was applied over their eyes to protect them from over-dryness. Mice were then fully constrained in a stereotaxic frame. A small 15-mm incision was made along the midline of the head and the orbicularis oculi muscle was gently dissected and retracted away from the bone. Small cotton balls were packed into the orbital cavity to control bleeding, and the infraorbital nerve was located (approximately 5 mm deep within cavity). Animals randomly assigned to receive the TIC injury surgery underwent surgical placement of a 2-mm length of chromic gut suture (6–0). Chromic gut suture was inserted parallel to the edge and among the infraorbital nerve fibers adjacent to the maxillary bone infraorbital fissure where it can be observed adhered to the nerve when dissected at the end of the experiment. This is done in order to prevent the chromic gut suture from being lost in the orbital cavity, but not to pierce the entire infraorbital nerve. Mechanical allodynia is induced in the mouse whisker pad due to the physical stimulation of the nerve by the suture as well as due to the irritative chromate salt released from the suture. Animals assigned to receive sham surgical procedures did not have the chromic gut suture placement, but only received the skin incision and muscle retraction. Naive animals did not receive any surgery.

Behavioral tests

All behavioral tests were conducted during the animals' active cycle (i.e. dark phase of the dark/light cycle) during the hours of 8:00 am to 6:00 pm. During testing, either a red-light or a dim lamp was illuminated to allow light for the experimenters. The light–dark preference and open field tests were repeated 4 weeks apart, and the elevated plus maze was given only once to avoid over-testing the mice (Walf and Frye, 2007).

Assessment of mechanical allodynia. Mechanical threshold of the whisker pad was measured before and after surgery with a modified up/down method using a graded series of von Frey fiber filaments (force: 0.008 g (size: 1.65); 0.02 g (2.36); 0.07 g (2.83); 0.16 g (3.22); 0.4 g (3.61); 1.0 g (4.08); 2.0 g (4.31); 6.0 g (4.74); Stoelting, Wood Dale, IL, USA). One experimenter gently restrained the mouse in their palm (2–5 min) with a cotton glove until the mouse was acclimated and calm. A second experimenter applied the von Frey filaments to the mouse's whisker pad. The 0.16-g (3.22)