J Oral Res
oral and craniofacial sciences

Article

Multidimensional study of orofacial chronic neuropathic pain: An experimental study in rats.

Claudia Daniela Montes-Angeles,[1] Nadia Gutierrez-Castañeda,[1] Christian Sosa,[1] Juan Jiménez,[2] Florencio Miranda[2] & Isaac Obed Pérez-Martinez.[1]

Affiliations: [1]Laboratorio de Investigación Odontológica, Facultad de Estudios Superiores Iztacala. Universidad Nacional Autónoma de México, México. [2]Laboratorio de Neurofarmacología Conductual, Unidad Interdisciplinaria en Ciencias de la Salud y la Educación. Facultad de Estudios Superiores Iztacala. Universidad Nacional Autónoma de México, México.

Corresponding author: Isaac O. Pérez-Martínez. San Sebastián Xhala, 54714 Cuautitlán Izcalli, México. Phone: (01-52) 5558705701. E-mail: isaac.perez@unam.mx, chac_opm@hotmail.com

Receipt: 09/11/2017 **Revised:** 10/03/2017
Acceptance: 10/23/2017 **Online:** 10/23/2017

Conflict of interests: None.

Ethics approval: Ethics Committee of the School of Higher Studies Iztacala of the Universidad Nacional Autónoma de México.

Funding: This project was financed by UNAM DGAPA PAPIIT RA203716 and FESI-DIP-PAPCA-2016-12

Authors' contributions: All authors collaborated with this work. All authors read and approved the final manuscript.

Acknowledgements: None.

Cite as: Montes-Angeles CD, Gutierrez-Castañeda N, Sosa C, Jimenez J, Miranda F & Pérez-Martínez IO. Multidimensional study of orofacial chronic neuropathic pain: An experimental study in rats. J Oral Res 2017; 6(10):270-275. doi:10.17126/joralres.2017.076

Abstract: Orofacial neuropathic chronic pain (NCP) is frequently attributed to lesions caused by orofacial surgeries and dental treatments. There are many experimental models available to study orofacial NCP, however, many are extremely painful for the animal due to the amplitude of the innervated region. A previously proposed mental nerve constriction model, mNC, was used in this project. Forty Wistar rats were randomly divided into two groups: one group included rats with mNC (n=20), and another rats with sham lesions (n=20). Through the use of the fixed ratio program and the progressive program, a decrease of motivation for a sweet substance, caused by the lesion, was evaluated. The possibility of alterations in cognitive learning and adaptation abilities was also assessed using the go/no-go behavioral task. The mNC group showed low induced and spontaneously evoked pain responses, as well as a decrease in the motivation for sucrose, a sign of anhedonia. This decrease does not depend on taste processing. Finally, although no alterations in the learning-memory process were observed, the mNC group did show alterations when adapting to a new rule.

Keywords: neuralgia, facial pain, animal experimentation.

INTRODUCTION.

Frequently, trigeminal nerve damage is attributed to surgeries and dental treatments.[1,2] These lesions result in the development of chronic neuropathic pain (NCP), which considerably diminishes the quality of life of the patients.[3] Two main emotional and cognitive sequelae asssociated with NCP are depression or anhedonia and the inability to adapt to enviromental challenges.[4-8]

To allow an integral therapeutic approach, all sensations, including pain, must be assessed from three main dimensions: the sensory dimension, the cognitive dimension, and the emotional or hedonic dimension.[9] The models to study NCP are important to discover pathological processes and to search for targets aimed at the control of orofacial NCP. The use of study models seeks to reproduce pain dimensions for human extrapolation and translational research.[10-15]

The ideal study should contain:

a) the evaluation of spontaneous and evoked behaviors associated with the nociceptive response, b) evaluation of the development of anhedonia or conducts that signal depression, and c) the development of cognitive deficits.

The use of chronic constriction of the mental nerve (mNC) has been

ISSN Online 0719-2479 - www.joralres.com © 2017

previously proposed as a model to study orofacial NCP in rats,[16] but it has not been fully characterized. This model induces spontaneous and evoked nociceptive responses to a lesser extent than other models. The tactile allodynic response generated by constriction of the mental nerve is due to a smaller area being innervated from this branch compared to other branches of the trigeminal nerve. Surgical access is simple, so the rodent is affected to a lesser degree. Despite a lower degree of pain provoked than in the use of other models, the lesion is sufficient to generate anhedonia and cognitive deficits.

The aim of this study is to evaluate the lesion of the mental nerve as a model for the study of orofacial chronic neuropathic multidimensional pain.

MATERIALS AND METHODS.

Animals

All procedures were carried out in accordance with the standards set by the Ethics Committee of the School of Higher Studies Iztacala of the Universidad Nacional Autónoma de México.

Fifty male Wistar rats with an initial weight of 200 to 250g were housed in transparent polycarbonate boxes held in a room at 21°C under a light and darkness cycle of 12-12 hours. The rats were randomly divided into an experimental group (n=20), a control group (sham) (n=20) and a group of 10 of rats that were left intact.

This last group (intact rats) was only used for the evaluation with mechanical stimulation to rule out the effect of an apparent surgery (sham) on the development of allodynia and hyperalgesia.

Chronic constriction of the mental nerve

A unilateral dissection was performed on the facial skin above the edge of the mandible, between the first molar and the incisor, and in front of the insertion of the masseter muscle to expose the mental nerve. Once localized, its constriction was achieved by a ligation performed with 5-0 catgut sutures. Subsequently, the incision was sutured.

In the rats of the control group, the same incision was made. However, the mental nerve was observed without performing any type of ligation, and the incision was sutured as well. Both surgeries were performed only on the right side.

Evaluation of orofacial spontaneous responses

The rats were individually placed in transparent plastic boxes with mirrors on their back walls and were recorded for 10 minutes. The recordings were analyzed with a double blind method to evaluate spontaneous orofacial responses, differentiating them from grooming behavior.[11]

Mechanical hypersensitivity test

Five von Frey filaments (10, 15, 26, 60 and 100gf) were applied to the areas innervated by the mental nerve and the responses were categorized on a previously proposed scale 0 to 4.[11] The stimuli were applied bilaterally and upwards.

Self-administration of sucrose

The animals were deprived of water at the beginning of the experiment until the stimulus-response behavior was obtained, a reward of a 10% sucrose solution was obtained at the response by pressing a lever.

The tests were performed in operant conditioning chambers using Med-PC automated software (MED Associates Inc., USA). Fifteen days following the lesion procedure, the rats were deprived of water for 12 hours, then placed in operant conditioning chambers for 40 minutes in a fixed ratio (FR) program, during which they received reinforcement after a fixed number of responses (a certain number of responses corresponded to a reward). The rats had a 40-minute training session in a FR2 program (2 responses for a reward) during 2 days, followed by 2 days in a FR 5 program. Then, the sessions were changed to progressive ratio (PR2),[17] increasing the number of required responses after each reinforcement given. The next day, PR5 was performed for the same length of time.

Preference for two bottles

The experiment consisted of placing two drinkers for each rat: one with water and the other containing a 10% sucrose solution. This experiment was carried out having *ad libitum* consumption of water and food, performing measurements after 24, 48 and 96 hours, and in sessions of 20 minutes. After 24 hours of consumption, the position of the bottles was changed.

Self-regulation model (go/no-go)

The rats, which were deprived of water for 12 hours, were placed in the operant chambers and exposed to two tasks. An adaption of the go/no-go task was used to measure self-regulation.[18] The task lasted 24 minutes and

was divided into 3-minute cycles during which the front light of the chamber was on ("on" cycles) interchanging with cycles of the same duration in which the light was off ("off" cycles), starting with the light on cycles .

The animals received a reward (one drop of 10% sucrose solution) for each four responses. They were rewarded only when the front light of the chamber was lit.

Reverse learning

To evaluate the ability to learn a new rule, the rats did a task called reverse learning, which consisted of the same elements as the go/no-go task. However, in this task the rats were only rewarded when the front light was off, not when it was on. This process was performed in 5 sessions.

Statistical Analysis

MatlabR2017a® (MathWorks, USA) was used to analyze the data. The statistical tests used for the analysis were: the *t*-test of independent groups, the one-way ANOVA and the Tukey test and Student's t-test. Significance was set at $p<0.05$ for all behavioral tests.

The coefficient of variation 2 was calculated as follows: $CV2\_IPI = [2(IPI_2 - IPI_1)/(IPI_2 + IPI_1)]$, where IPI is the interval between the responses.

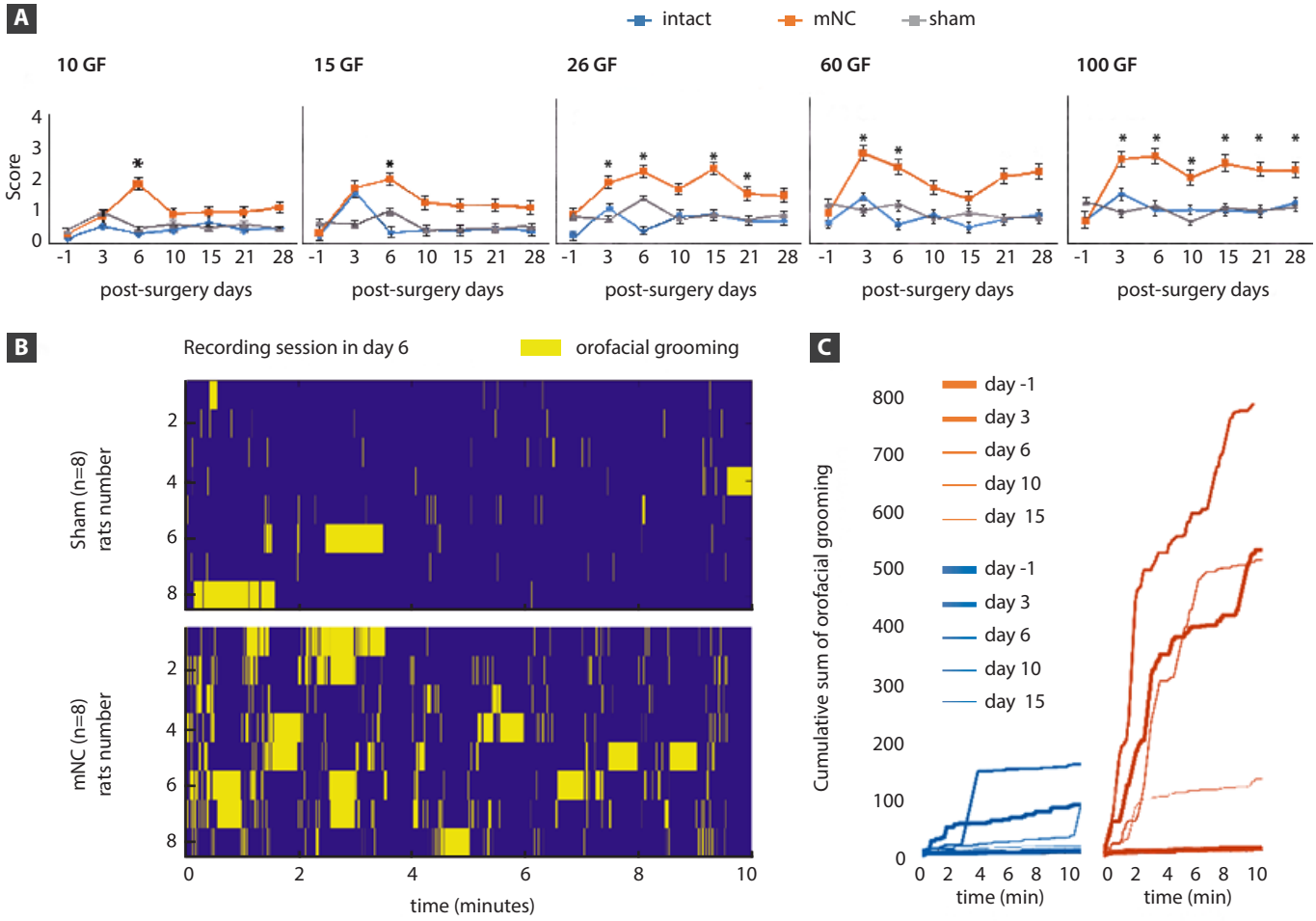
RESULTS.

Figure 1 shows the results of the evaluation of the sensory dimension after mNC.

Figure 2 shows the evaluation of the hedonic dimension of orofacial pain induced by mNC.

Figure 3 shows the evaluation of learning processes associated with the adaptation to a new rule.

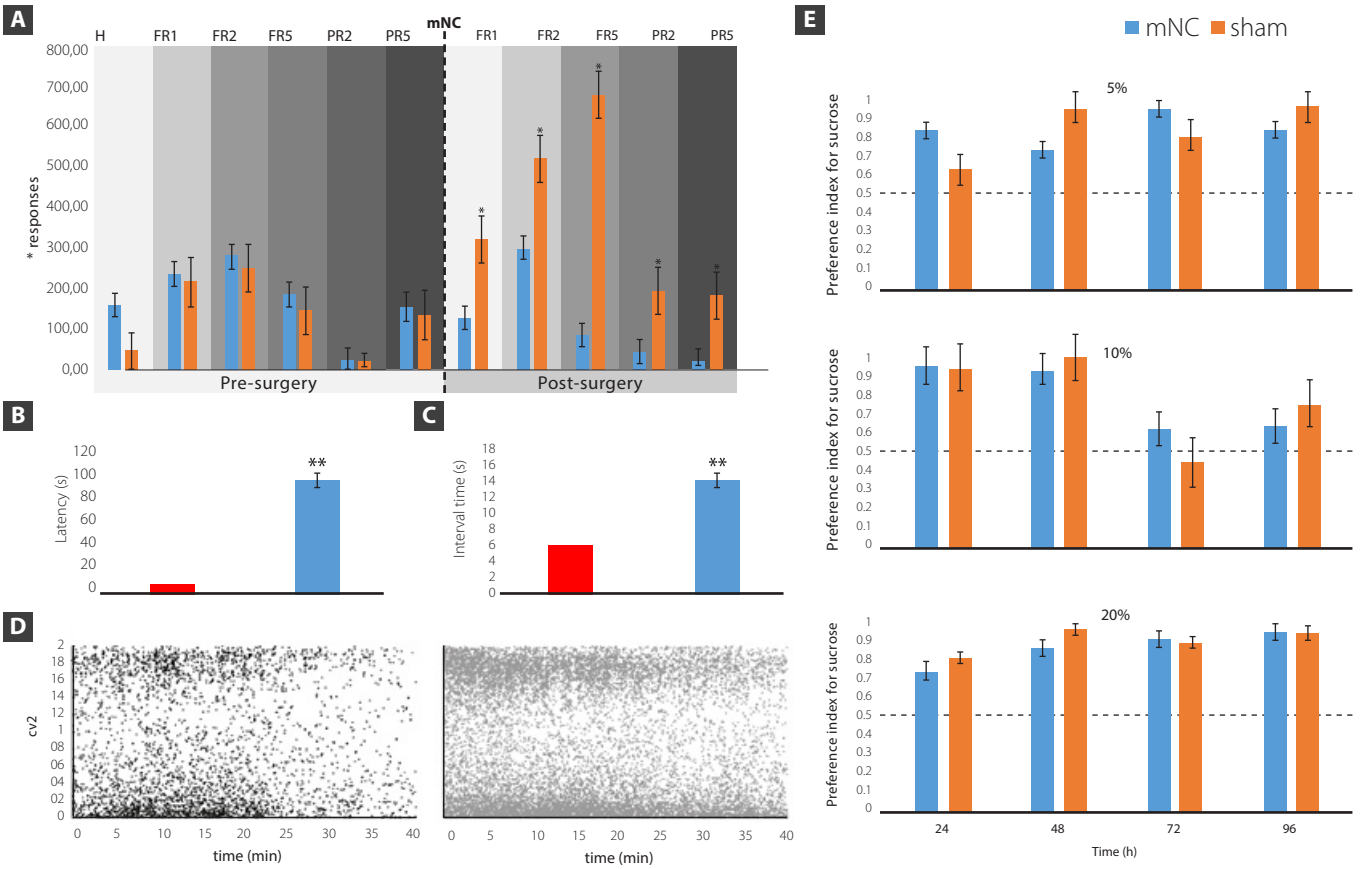
Figure 1. Evaluation of the sensorial dimension after mNC.



A. The nociceptive response (Score 0-4) to the application of different forces (grams-force) and in different post-surgical days is shown.
B. Graphic representation of spontaneous orofacial grooming (yellow lines) of the control group (upper panel) and mNC group (lower panel), the data are organized rat by rat for post-surgery day 6.
C. Cumulative sum of orofacial grooming time at post-lesion days -1, 3, 6, 10 and 15 for the control group (left panel) and experimental group (right panel), being the maximum peak of spontaneous grooming on day 6, after which the grooming gradually decreases.
*$p<0.05$; **$p<0.001$.

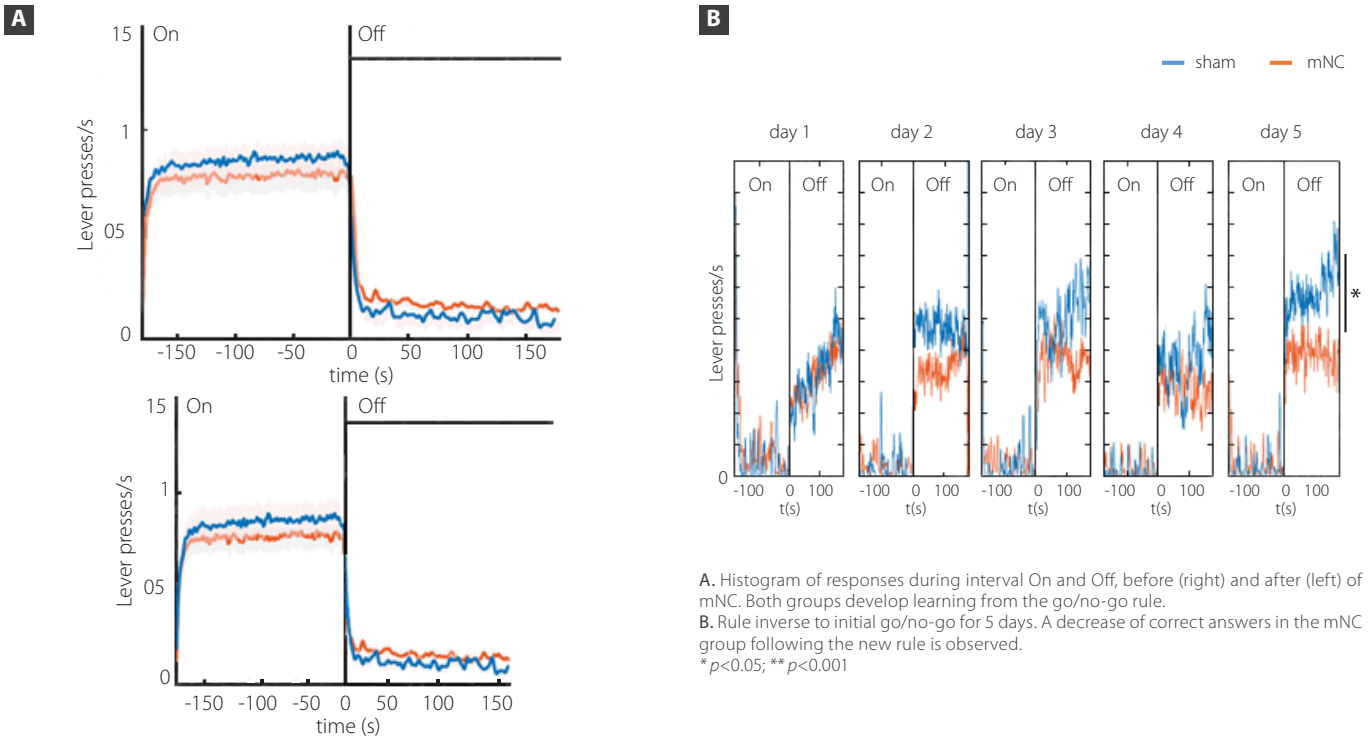
ISSN Online 0719-2479 - www.joralres.com © 2017

Figure 2. Evaluation of the hedonic dimension of orofacial pain induced by mNC.



A. mNC produces a decrease in sucrose motivation in the fixed and progressive ratio programs (FR, FR2, FR5, PR2 and PR5) compared to the control group.
B. Latency to the first response in the behavioral session.
C. Average in the time interval between responses.
D. Calculation of coefficient of variation 2 of response interval; the left panel represents the mNC group and the right panel the control group (sham), the temporal pattern of responses is very similar and independent of the number of them.
E. Preference Index for sucrose at different concentrations in the test protocol of two bottles.
*$p<0.05$; **$p<0.001$.

Figure 3. Evaluation of learning processes associated with the adaptation to a new rule.



A. Histogram of responses during interval On and Off, before (right) and after (left) of mNC. Both groups develop learning from the go/no-go rule.
B. Rule inverse to initial go/no-go for 5 days. A decrease of correct answers in the mNC group following the new rule is observed.
*$p<0.05$; **$p<0.001$

DISCUSSION.

The study of pain requires models that replicate the sensory, emotional and cognitive demands. The search for these behavioral correlates at experimental level is one of the most important challenges of the biomedical sciences. This would allow the application of specific therapies and the search for therapeutic targets to control orofacial pain integrally.[19]

Most models of orofacial NCP are based only on motor reflexes associated with tactile stimuli.[10,11,15] In other models of trigeminal lesions, particularly of the infraorbital branch; the analysis of evoked responses is also used as a sign of spontaneous pain. It is necessary to observe both the evoked and spontaneous responses following the neuropathic lesion for the analysis of the sensory dimension.

In the mNC model, the responses observed to be induced by the application of forces directly to the skin of the rat were sufficient to generate nocifensive responses, compared to a previous report in which the level of response was much lower.[11] This is due to the fact that the mental nerve innervates a small area of the orofacial region; therefore, the degree of pain is likely to be less. Consequently, the use of this model to study the cognitive or emotional alterations induced by chronic constriction of the mental nerve is acceptable after 15 days, at which time spontaneous grooming in no longer present.

The pain will not influence variables, so a recovery period of 15 days following the lesion is proposed. Using this model, the motivation for a sweet sucrose solution to study the development of anhedonia was measured. Two reference points were taken: the first was the "latency point", which is the time it takes the rat to press the lever for the first time in each experiment; the second was the so-called "break point", which is defined as the number of responses at the end of the experiment, before the rats stop responding for a fixed period of time. The justification for this measure is that the break point is presumed to reflect that the effort required to obtain the next reward has become too large, so the rats stop responding.[17]

It was observed that the group with trigeminal lesion presented lower motivation to obtain a sweet solution and did not depend on changes in gustatory perception or motor response.

Finally, deficiency in environment adaptation plays a very important role in pathophysiology or orofacial pain among the cognitive capacities. Using an adaptation of the go/no-go task previously used for infraorbital nerve lesion, it was observed that learning and memory were not altered by the mental nerve lesion.[18] This study added the learning of a new rule in this study model, observing, however, that 5 days after the learning of the new rule the mNC group could not adapt completely compared to the control group.

In this article, the use of tools for the study of orofacial NCP was proposed. This model was gentler for the rodents, as it was less invasive and generated a lower degree of spontaneous and provoked pain. It also allowed the study of the affective and cognitive dimension of pain for subsequent studies for therapeutic alternatives applied to the control of orofacial NCP.

REFERENCES.

1. Tınastepe N, Oral K. Neuropathic pain after dental treatment. Agri. 2013;25(1):1–6.
2. Fukuda K, Ichinohe T, Kaneko Y. Pain Management for Nerve Injury following Dental Implant Surgery at Tokyo Dental College Hospital. Int J Dent. 2012;2012:209474.
3. Dogru Huzmeli E, Melek I. Neuropathic pain's biopsychosocial effects. Neurol Sci. 2017
4. Zis P, Daskalaki A, Bountouni I, Sykioti P, Varrassi G, Paladini A. Depression and chronic pain in the elderly: links and management challenges. Clin Interv Aging. 2017;12:709–20.
5. Vinall J, Pavlova M, Asmundson GJ, Rasic N, Noel M. Mental Health Comorbidities in Pediatric Chronic Pain: A Narrative Review of Epidemiology, Models, Neurobiological Mechanisms and Treatment. Children. 2016;3(4):pii: E40.
6. Doan L, Manders T, Wang J. Neuroplasticity underlying the comorbidity of pain and depression. Neural Plast. 2015;2015:504691.
7. Bushnell MC, Case LK, Ceko M, Cotton VA, Gracely JL, Low LA, Pitcher MH, Villemure C. Effect of environment on the long-term consequences of chronic pain. Pain. 2015;156(Suppl 1):S42-9.
8. Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, Freeman R, Truini A, Attal N, Finnerup NB, Eccleston C, Kalso E, Bennett DL, Dworkin RH, Raja SN. Neuropathic pain. Nat Rev Dis Primers. 2017;3:17002.
9. Wijma AJ, van Wilgen CP, Meeus M, Nijs J. Clinical biopsychosocial physiotherapy assessment of patients with chronic pain: The first step in pain neuroscience education. Physiother Theory Pract. 2016;32(5):368–84.
10. Ding W, You Z, Shen S, Yang J, Lim G, Doheny JT, Chen L, Zhu S, Mao J. An Improved Rodent Model of Trigeminal Neuropathic Pain by Unilateral Chronic Constriction Injury of Distal Infraorbital Nerve. J Pain. 2017;18(8):899–907.
11. Deseure K, Hans GH. Chronic Constriction Injury of the Rat's

ISSN Online 0719-2479 - www.joralres.com © 2017

Infraorbital Nerve (IoN-CCI) to Study Trigeminal Neuropathic Pain. J Vis Exp. 2015;(103):e53167.
12. Deseure K, Hans G. Behavioral study of non-evoked orofacial pain following different types of infraorbital nerve injury in rats. Physiol Behav. 2015;138:292–6.
13. Lynds R, Lyu C, Lyu GW, Shi XQ, Rosén A, Mustafa K, Shi TJS. Neuronal plasticity of trigeminal ganglia in mice following nerve injury. J Pain Res. 2017;10:349–57.
14. Ma F, Zhang L, Oz HS, Mashni M, Westlund KN. Dysregulated TNFα promotes cytokine proteome profile increases and bilateral orofacial hypersensitivity. Neuroscience. 2015;300:493–507.
15. Challa SR. Surgical animal models of neuropathic pain: Pros and Cons. Int J Neurosci. 2015;125(3):170–4.
16. Grelik C, Bennett GJ, Ribeiro-da-Silva A. Autonomic fibre sprouting and changes in nociceptive sensory innervation in the rat lower lip skin following chronic constriction injury. Eur J Neurosci. 2005;21(9):2475–87.
17. HODOS W. Progressive ratio as a measure of reward strength. Science. 1961;134(3483):943–4.
18. Kniffin TC, Danaher RJ, Westlund KN, Ma F, Miller CS, Carlson CR. Persistent neuropathic pain influences persistence behavior in rats. J Oral Facial Pain Headache. 2015;29(2):183–92.
19. Carlson CR. Psychological considerations for chronic orofacial pain. Oral Maxillofac Surg Clin North Am. 2008;20(2):185–95.