



## Research Paper

## Effect of chronic stress on capsaicin-induced dental nociception in a model of pulpitis in rats

Maryam Raof<sup>a,b</sup>, Ehsan Ashrafganjoui<sup>a</sup>, Raziieh Kooshki<sup>c</sup>, Mehdi Abbasnejad<sup>c</sup>, Jahangir Haghani<sup>b</sup>, Sara Amanpour<sup>d,\*</sup>, Mohammad-Reza Zarei<sup>d</sup>

<sup>a</sup> Endodontology Research Center, Kerman University of Medical Sciences, Kerman, Iran

<sup>b</sup> Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran

<sup>c</sup> Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran

<sup>d</sup> Oral and Dental Diseases Research Center/Kerman Social Determinants on Oral Health Research Center, Kerman University of Medical Sciences, Kerman, Iran

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## ABSTRACT

**Objective:** Chronic stress can alter nociceptive sensitivity. However, the effect of stress exposure on dental nociception has been less addressed. Therefore, the present study investigated the effects of chronic exposures to some social and psychological stresses on pulpal nociceptive responses.

**Design:** The stress groups were constructed as follows: forced swimming (n = 6), restraint (n = 6), and mild (n = 10) and severe (n = 15) crowding stresses. Rats were subjected to stress for 1 h per day for a week. At the end of the stress session, pulp irritation was induced by intradental application of capsaicin (100 µg). There were another capsaicin or capsaicin plus stress training groups that received articaine 5 min before the administration of capsaicin. Nociceptive responses were recorded for 40 min. The time (in s) of continuous shaking of the lower jaw and excessive grooming and rubbing of the mouth near the procedure site was measured as nociceptive behaviors. Data was analyzed using one-way analysis of variance (ANOVA) followed by post hoc Tukey's test.

**Results:** Significant nociceptive responses were evoked by the administration of capsaicin. Exposures to forced swimming (p < 0.01), restraint (p < 0.001), and both mild and severe crowding stresses (p < 0.05) exaggerated capsaicin-induced nociceptive reaction. There was, however, no significant difference in nociceptive reaction time between the different stress groups. Articaine buccal infiltration attenuated nociceptive time in capsaicin and capsaicin plus stress training groups (p < 0.001).

**Conclusions:** The current data support the association between chronic stress exposures and nociceptive behavior following intradental capsaicin administration.

## 1. Introduction

Dental pain arising from pulpitis is one of the major orofacial problems associated with significant neural deficiency and social disabilities (Sebring, Dimenäs, Engstrand, & Kvist, 2016; Sheiham, 2005). The dental pulp has a highly complex structure that is innervated by myelinated A and unmyelinated polymodal C fibers. Neuropeptide-containing nerves have also been found in the dental pulp. They synthesize and release sensory neuropeptides such as calcitonin gene-related peptide (CGRP) and substance P in response to noxious stimulations (Caviedes-Bucheli, Lombana, Azuero-Holguín, & Munoz, 2006; Manuja, Nagpal, Pandit, & Chaudhary, 2010). A number of sensory nerve fibers innervating human pulp express transient receptor potential vanilloid-1 (TRPV1) as a nonselective ligand-gated cation channel. TRPV1 can be activated in response to various types of stimuli

including capsaicin, a bioactive compound of cellie paper (Chung & Oh, 2013). Activation of TRPV1 leads to burning and sturdy pain responses via cytosolic calcium increase and a subsequent induction of pro-inflammatory sensory neuropeptides in sensory neurons (Gouin et al., 2017; Kárai, Russell, Iadarola, & Oláh, 2004).

Stimulation of trigeminal nerves by capsaicin is a reliable experimental model for the study of pathophysiologic features of orofacial nociceptive responses in rat. Injection of capsaicin into the vibrissa pad of rat is associated with distinctive patterns of nociceptive responses (Pelissier, Pajot, & Dallel, 2002). Moreover, intradental administration of capsaicin causes significant dental inflammatory response in rats (Chidiac et al., 2002; Raof, Esmaeili-Mahani, Nourzadeh et al., 2015). Capsaicin-stimulated CGRP release from dental pulp has been introduced as a pre-clinical model for the study of peripheral neuropeptide secretion in healthy tissue (Fehrenbacher, Sun, Locke,

\* Corresponding author at: Department of Oral and Maxillofacial Pathology, School of Dentistry, Shafa St, Jomhoori Eslami Blvd, Kerman, Iran.  
E-mail address: [saraamanpour@gmail.com](mailto:saraamanpour@gmail.com) (S. Amanpour).

Henry, & Hargreaves, 2009).

Stress is an adaptive response to interruptions in normal physiologic state. It prompts a variety of neuroendocrine and immunological responses to maintain internal homeostasis (McEwen, 2007). Prolonged stresses are associated with structural and neurophysiological changes in brain (Czéh et al., 2001; McEwen et al., 2015). Interestingly, there is strong evidence to suggest that exposure to stress challenges may change the intensity of pain (Crettaz et al., 2013; Long, Sadler, & Kolber, 2016). The associations between stressful situations and perception of trigeminal nociception were also investigated in some clinical and preclinical studies (Bergamini et al., 2017; Nevalainen et al., 2016). Psychological stress has been shown to be associated with increased temporomandibular joint-evoked responses (Okamoto, Tashiro, Chang, Thompson, & Bereiter, 2012). However, it has been reported that acute restraint stress can diminish formalin-induced temporomandibular joint nociceptive responses in female rats (Botelho, Gameiro, Tuma, Marcondes, & Ferraz de Arruda Veiga, 2010).

As mentioned above, it is well established that stress can alter orofacial nociceptive responses. However, stressor effects on the perception of pulpal nociception have received less attention. In the current study, by using forced swim, restraint, and social crowded test as animal models of social and psychological stress, the possible effects of chronic stress on capsaicin-evoked pulpal nociception was investigated in rats.

## 2. Materials and method

### 2.1. Animals

The experiments were performed on adult male Wistar rats (230–270 g). Animals were housed in a controlled room environment ( $23 \pm 1^\circ\text{C}$ ) and kept on a standard 12:12 h light/dark cycle. Food and water was provided ad libitum. The experimental protocol was approved by the Ethical Committee of Kerman University of Medical Sciences, Kerman, Iran. The rats were adapted to the laboratory environment in their home cages 1 h per day for 2 weeks prior to the initiation of any procedure manipulation and behavioral assessment.

### 2.2. Experimental groups

The rats were randomly divided into various experimental groups as follows: intact group ( $n = 6$ ) which received no injection; capsaicin group ( $n = 6$ ), which received a small cotton pellet dampened with capsaicin solution (100  $\mu\text{g}$ ) into a prepared cavity on the left mandibular incisor; capsaicin plus articaine-treated rats ( $n = 6$ ), which received 0.1 mL of 4% articaine hydrochloride in the alveolar mucosa near the apex of the tooth prior to capsaicin administration; and different experimental stress groups including forced swimming ( $n = 6$ ), restraint ( $n = 6$ ), and mild ( $n = 10$ ) and severe ( $n = 15$ ) crowding stresses groups that also received cotton pellet dampened with capsaicin solution after exposure to stress. Moreover, to elucidate stress effects of nociceptive induction, the same stress groups that received no capsaicin injection were used. The calculation of an adequate sample size was based both on previous reports (Abbas, Naqvi, Mehmood, Kabir, & Dar, 2011; Gameiro et al., 2005; Hadigol & Rajaei, 2011) and a pilot study.

### 2.3. Forced swimming test

Forced swimming test is one of the most commonly used method for the assessment of antidepressant-like behavior and stress responses in rodents (Armario, Gavaldà, & Martí, 1995). Each rat was placed in a Plexiglas container in a cylinder (60 cm high  $\times$  12 cm wide) filled to 50 cm with water at a temperature of  $22 \pm 1^\circ\text{C}$ . Rats were allowed to swim 5 min per day for seven successive days. At the end of the test, rats were removed from the container and lightly dried.

### 2.4. Restraint stress test

Restraint stress is well known as a useful method to investigate neurophysiological features of stress (Gregus, Wintink, Davis, & Kalynchuk, 2005). In the present study, the rats were restrained 1 h per day for a week in a plastic conical tube with 5 cm diameter and 12 cm height. The tube was fixed with ventilation holes, and it was plugged so that animals were unable to move/turn around.

### 2.5. Crowding stress test

Crowding stress is an imposed movement limitation, which is considered a psychosocial stress (Blanchard, McKittrick, & Blanchard, 2001). In this study, before being exposed to stress, the animals were kept in groups of five per cage (40  $\times$  20  $\times$  20 cm). To induce crowding stress, two groups of animals consisting of 10 (mild stress condition) and 15 (severe stress condition) rats per cage were used. The sample size calculation was conducted on the basis of both previous publications and a pilot study (Hadigol & Rajaei, 2011). Each groups exposed to crowding 1 h per day for 1 week. Following the stress cessation, animals were returned to their home cages.

### 2.6. Induction and management of nociceptive responses

On the day of testing for nociceptive behavior, rats were taken to the testing room and habituated for 30 min. After the induction of short-term anesthesia with carbon dioxide ( $\text{CO}_2$ ), a cavity ( $2 \times 2 \times 2 \text{ mm}^2$ ) was prepared in the gingival one-third of the distal aspect of left mandibular incisors using a small fissure bur in a high-speed handpiece with water coolant. With the help of magnification ( $2.5\times$ ), pulp exposure was prevented. A small cotton pellet dampened with capsaicin solution (100  $\mu\text{g}$ ) was left in the cavity under a light-cured glass-ionomer (Fuji II, GC, Japan) restoration.

Upon capsaicin administration, each rat was placed in the transparent Plexiglass container (30  $\times$  30  $\times$  30 cm) with a mirror positioned at a  $45^\circ$  angle below the floor to allow unbarred observation of the animals. The nociceptive responses were monitored using a digital video camera for 40 min. The measured parameter for nociceptive behavior was the cumulative time (seconds) spent in continuous shaking of the lower jaw, excessive grooming, and rubbing of the mouth near the procedure site (Raoof, Esmaeili-Mahani, Abbasnejad et al., 2015). In the orofacial capsaicin test for rats, the nociceptive response peaks about 15 min after the administration of capsaicin and then gradually decreases (Pelissier et al., 2002). Therefore, the time course of nociceptive behavioral response was divided into eight blocks of 5 min to specify distinctive patterns of capsaicin-induced nociceptive behaviors. Following the assessment of nociceptive responses, the rats were euthanized by exposure to high concentrations of  $\text{CO}_2$ .

### 2.7. Statistical analysis

Data are presented as means  $\pm$  standard error of mean. Statistical analysis of nociceptive behavior was performed using one-way analysis of variance (ANOVA) followed by post hoc Tukey's test. Differences were considered significant if  $p < 0.05$ .

## 3. Results

### 3.1. Orofacial capsaicin test

Intradental application of capsaicin produced nociceptive behavior that was characterized by shaking of the lower jaw, scratching, and rubbing of the injected site. The highest nociceptive response time was recorded within 10–15-min intervals following capsaicin administration. The cumulative nociceptive time during 40 min was  $856 \pm 13.1 \text{ s}$  (Fig. 1).

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