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Psychological stress may contribute to temporomandibular joint disorder in rats

Gaoyi Wu, PhD,[a,1] Lei Chen, PhD,[b,1] Huang Fei, PhD,[c,1] Yucheng Su, PhD,[d] Guoxiong Zhu, PhD,[a,**] and Yongjin Chen, PhD[c,*]

[a] Department of Stomatology, Jinan General Military Hospital, Jinan, China
[b] Department of Orthodontics, Jinan Stomatological Hospital, Jinan, China
[c] Department of General Dentistry and Emergency, School of Stomatology, Fourth Military Medical University, Xi'an, China
[d] Department of Stomatology, Peking Union Medical College Hospital, Beijing, China

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ABSTRACT

Background: Psychological stress is considered a possible pathogenic factor for temporomandibular joint disorders (TMJD), but few reports have supplied direct evidence. This study was designed to observe the effects of psychological stress on the masticatory muscles and condylar processes in rats to directly investigate the role of psychological stress in TMJD morbidity.
Materials and methods: A well-established rat communication box model was used to compare the myoelectric profiles of temporal and masseter muscles and condylar microstructure among rats in a control group, a psychological stress group (PS group), and a diazepam (anxiolytic agent) injection group (PS + DI group). Reverse-transcription polymerase chain reaction was also used to analyze the substance P mRNA and calcitonin gene–related peptide mRNA levels expressed in condylar cartilages during different phases of psychological stress.
Results: At 1, 3, and 5 wk, both temporal and masseter muscles in the PS group exhibited a significantly higher electrical potential in relaxation than those in the control group ($P < 0.01$). The electrical potential during contraction of the temporal and masseter muscles was higher than in the relaxation or control group at 1, 3, and 5 wk ($P < 0.01$). Scanning electron microscopy demonstrated pathologic changes in condylar processes in the PS group that were not observed in the PS + DI group. Reverse-transcription polymerase chain reaction also showed that the expression of substance P and calcitonin gene–related peptide in rat temporomandibular joint was upregulated during each phase of the psychological stress ($P < 0.05$).
Conclusions: Psychological stress may play an important role in the formation of TMJD.

1. Introduction

Temporomandibular joint disorders (TMJD) are characterized by temporomandibular and/or peritemporomandibular pain, temporomandibular clicks, and limited mouth opening, which can impair sleep and quality of life. The etiology is unclear, and no ideal treatment exists [1]. Psychological stress is a risk factor for TMJD [2–5]. Because of difficulties in model establishment, parameter quantification, and the diversity of influencing factors in experimental evaluations [6,7], the

* *Corresponding author*. Department of General Dentistry and Emergency, School of Stomatology, Fourth Military Medical University, Xi'an, China. Tel./fax: +0086 02984776488.
** *Corresponding author*. Department of Stomatology, Jinan General Military Hospital, Jinan, China. Tel./fax: +0086 053151665829.
E-mail addresses: dentist_effendi@yahoo.cn (G. Zhu), fmmugaoyi@hotmail.com (Y. Chen).
[1] Gaoyi Wu, Lei Chen, and Huang Fei contributed equally to this paper and should be regarded as co–first authors.
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definite mechanism underlying psychological stress and TMJD appears to be equally complex and has not yet been confirmed, and most experimental studies are focused on observational and/or epidemiologic studies.

Two pain neurotransmitters, substance P (SP) and calcitonin gene–related peptide (CGRP), are extensively distributed in the temporomandibular joint (TMJ) and are associated with the generation and development of TMJD [8]. As SP and CGRP are the main mediators that lead to pain in local inflammation, these neurotransmitters are implicated in the damage to articular chondrocytes [9,10].

The hypothesis of the present study was that psychological stress could alter masticatory myoelectricity, TMJ microstructure, and SP and CGRP levels. Using an emotional stress paradigm, which employed intraspecies psychological communication within a communication box [11], we induced experimental psychological stress in rats and then tested our hypothesis on related parameters, such as the myoelectricity of masseter muscles, the microstructure of TMJ, and expression of SP and CGRP in TMJ. The present study sought to investigate the correlation between psychological factors and TMJD *via* evaluation of changes in masticatory myoelectricity, TMJ microstructure, and SP and CGRP levels in rats pre- and post-psychological stress induced by a stress model.

2. Materials and methods

2.1. Animals

A total of 120 grade one male Sprague-Dawley rats, weighing 150 ± 10 g, were supplied by the Laboratory Animal Center of Fourth Military Medical University (certificate number: SCXK J 2005-008). All animal experiments were conducted under the supervision of experienced veterinary surgeons. The experimental protocol was approved by the Laboratory Animal Research Center of the Fourth Military Medical University and the study was conducted according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (8th edition) as published online at http://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf. As an acclimatizing and calming procedure, the immature rats were bought and fed before the beginning of the experiment in our study, considering the adaptability of rodents.

2.2. Instruments and devices

The major instruments and devices used in the present study were: (1) an electric stress generator with the following technical parameters: stress voltage (DC), 48 V; stress frequency, 0.5 Hz; (2) a rat communication box; (3) a DM3000B microscope (Leica, Solms, Germany); (4) a S3400 scanning electron microscope (Hitachi, Tokyo, Japan); (5) a TG16-WS low-temperature high-speed centrifuge device (Luxiang, Beijing, China); (6) a TC-96/G/H (b) PCR meter (Bioer, Beijing, China); (7) an EPS200 electrophoresis system (Tanon, Shanghai, China); and (8) an FR-980 ultraviolet transmittance and reflection analyzer (Furi, Shanghai, China). A technician supervised all the electrical apparatus to ensure that it worked normally in the experiment process.

2.3. Reagents

The following reagents were used: TaqDNA polymerase (2× Taq PCR; TIANGEN, Beijing, China); PCR primers (Kinst, Beijing, China); agarose (GeneTech, Shanghai, China); ethidium bromide (SBS, Dalian, China); and DNA restrictive endonuclease (Takara, Dalian, China).

2.4. Animal randomization

A total of 120 animals were categorized randomly into the following groups: blank control group, foot shock group (FS group), psychological stress group (PS group), and diazepam injection group (PS + DI group). Each of the four groups was assessed at week 1, week 3, and week 5 after psychological stress; 12 subgroups were obtained, with 10 rats each. The parameters of the animals, including body weight and sex ratio, were comparable between groups.

2.5. Experimental model establishment

This model was designed based on stress models reported by Rosales *et al.* [11] and Funada and Hara [12] The communication box had 16 chambers, each 40 × 40 × 20 cm, separated by transparent porous plastic plates, which were proved to provide electrical insulation by previous examination before the experiment. The plates prevented physical contact between animals but allowed them to receive visual, auditory, and olfactory cues from neighboring animals. All the rats' feet were examined before the experiment to ensure the foot skins were soft and without callus.

The chamber bottoms were stainless wire mesh, through which a foot shock could be given if the wire was electrified. These comprised a grid floor of 5-mm-diameter stainless steel rods placed at 0.3-cm intervals. A 48-V electric generator connected to the grid floor generated a foot shock every 2 s for PS and PS + DI rats. Plastic plates covered the bottom of eight of these chambers to prevent electric shocks, so in this experiment the PS and PS+DI rats do not receive shock.

Animals were individually confined and placed into each compartment of the communication box for 1 h daily without any stressors to acclimatize them to the new surroundings; this process lasted for 1 wk. During the period of model establishment, all animals were placed into the same compartment. Through this procedure, we could reduce the effect of new surroundings on the results. The current study predicts that because the walls of the chambers are transparent and porous, the animals in the PS + DI and PS group who do not receive foot shock are likely to experience psychological stress by witnessing the screaming and jumping of the animals in the FS group resulting from the electric shock, *via* visual, auditory, and gustatory routes [13]. All the parameters were set as, reported in the studies by Rosaleset al [11] and Funada and Hara [12], to make animals reach the state of terror but without visible body wound.

During the stress stimulation, the electric foot shock was introduced to the FS rats (stress senders) for 30 min/d at a fixed time (9:00–9:30 AM). At 8:30 AM daily, the PS + DI rats were injected with diazepam (10 mg; 2 mL, 1 mg/kg) to resist