Psychological stress induces alterations in temporomandibular joint ultrastructure in a rat model of temporomandibular disorder

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Objective. The objective of this study was to investigate the effects of psychological stress on temporomandibular disorder (TMD).

Study design. A communication box was used to induce psychological stress (PS) in rats. Then, the ultrastructure of temporomandibular was observed using scanning electron microscopy. Interleukin-1 (IL-1) and IL-6 were measured with reverse transcription polymerase chain reaction.

Results. The PS group showed evidence of ultrastructural changes in the condyle and articular disk after stimulation, i.e., incomplete gelatinlike material was observed on the condyle after 1 week of PS, wider waves on the articular disk and exposed condylar collagen were observed after 3 weeks of PS, and cracks were apparent on the surface of the condyle. The expression of IL-1 and IL-6 in the condyle cartilage significantly increased after exposure to psychological stress.

Conclusions. These results indicate that psychological stress induces ultrastructure alterations in the temporomandibular joint and plays an important role in TMD. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:e106-e112)**

Temporomandibular disorder (TMD), a functional disorder of the temporomandibular joint that is characterized by temporomandibular joint sounds, impaired movement of the mandible, limitation in mouth opening, preauricular pain, facial pain, jaw tenderness on function, and headaches, is an important area of investigation in dentistry. The etiology of TMD is now considered to be multifactorial, and psychological stress has been regarded as an important factor in the etiology and maintenance of TMD.[1,2]

Most studies of psychological stress on TMD are epidemiology reports, clinical case studies, or questionnaire surveys; well-controlled experiments have seldom been reported.[3-5]

One reason for this lack of data is that it has been difficult to establish research models of psychological stress that correlate with TMD. Moreover, there are many factors involved in the etiology of TMD, part of them are difficult to analyze quantitatively.[6] Researchers have shown that psychological stress can lead to symptoms, such as abnormal mandibular movements, pain, and facial muscle fatigue.[7] It was reported that psychological stress caused mitochondrial injuries and hyperemia of the masticatory muscle capillaries in rats. Furthermore, such stress can result in dramatic alterations in the energy metabolism of the masticatory muscles as reported in a recent study.[8]

In this study, we hypothesized that psychological stress could increase the serum concentration of cortisol and adrenocorticotropic hormone (ACTH), alter the ultrastructure of the temporomandibular joint (TMJ), and induce inflammation of the TMJ. With an emotional stress paradigm, which used intraspecies emotional communication within a communication box,[3,9,10] we introduced experimental correlates of psychological stress,

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such as anxiety and depression, in rats. We then tested our hypothesis by examining the TMJ of the animals that experienced psychological stress. Additionally, we included a negative control group, in which rats were administered the antianxiety drug diazepam before being exposed to psychological stress. This study sought to assess alterations in the serum concentration of cortisol and ACTH, the TMJ ultrastructure, and in the expression levels of interleukin-1 (IL-1) and IL-6 in the condyle cartilage of the TMJ in rats following exposure to psychological stress.

MATERIAL AND METHODS

The animal stress model used in this study was referenced by Rosales et al, Yamamoto et al, and Funada et al.[11-13] A total of 120 male, Wistar, albino rats were included in the study. The animals weighed between 140 and 160 g and were approximately 35 days old. The rats were housed in 80 × 45 × 40-cm cages in a temperature-controlled room at 24°C under a 12-hour light/dark cycle. They were given free access to food and water. Before the experiments, the rats were housed in a communication box for 1 hour a day for 5 days to allow them to acclimatize to the box. The rats were then randomly divided into the control (CON), foot-shocked (FS), psychological stress (PS), and psychological stress plus diazepam (PS+DI) groups. Each group included 30 rats. The durations of psychological stimulation were 1 week (1 wk), 3 weeks (3 wk), and 5 weeks (5 wk). Each group was divided into 3 subgroups (1 wk, 3 wk, and 5 wk). The FS, PS, and PS+DI rats were housed in 1 communication box during the procedure, as later in this article. The FS rats received an electric foot shock, the PS rats were subjected to psychological stress, and the PS+DI rats were given a subcutaneous injection of diazepam 30 minutes before being subjected to the psychological stress. The rats in the control group were housed in a second communication box under the same conditions, but they were not subjected to the electric foot shock or psychological stress. The study was performed twice. Thus, 30 animals were included per group, with 10 animals in each of the 3 subgroups.

After 1, 3, and 5 weeks of stimulation, blood was drawn from the ophthalmic artery of the 10 rats in the control, PS, and PS+DI groups. Serum samples were prepared to measure the serum stress indexes. Then, the 10 rats in each group were randomly divided into 2 subgroups, with 5 rats in each group. The first 5 rats in each subgroup were immediately killed with an intraperitoneal injection of an overdose of thiamylal sodium, and their bilateral TMJs were removed. The other 5 rats were housed in another communication box under the same conditions as the control group to observe their recovery. The recovery period was as long as the stimulation period. Thus, the recovery period was evaluated at 1 wk, 3 wk, and 5 wk. Following the recovery period, the 5 rats were killed to extract the TMJ samples. All of the TMJ samples were obtained according to guidelines established by the University Internal Review Board for the Use of Mouse Subjects. The experimental procedures were reviewed and approved by the Ethics Committee of the Fourth Military Medical University. The articular disk and condyle were dissected for ultrastructural observations using electron microscopy. Additionally, RNA was extracted from the condyle cartilage to investigate the expression of IL-1 and IL-6 using reverse transcription polymerase chain reaction (RT-PCR).

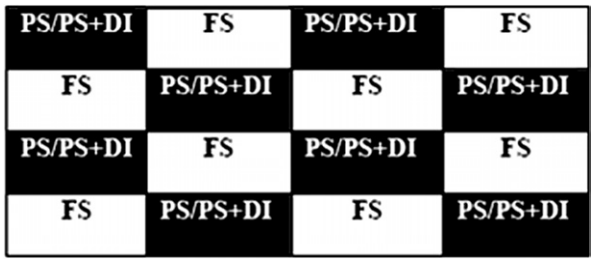

Fig. 1. Diagram representing the communication box. FS, foot-shocked; PS, psychological stress; PS+DI, psychological stress plus diazepam injection.

Animal model for psychological stress

The communication box[9,10,14] was selected as the psychological stress apparatus in this study. The box consisted of 16 compartments that were each 16 × 16 cm and were separated by transparent plastic boards with several small holes. The boards prevented the animals from making physical contact with each other, but allowed them to receive cues, such as visual, auditory, and olfactory sensations, from the neighboring animals. Each compartment was equipped with a grid floor of stainless steel rods, which were 5 mm in diameter and placed at intervals of 0.3 cm. A 48-V electric generator, which was made by the Department of Biomedical Engineering of the Fourth Military Medical University, was connected to the grid floor to produce an electric current and generate an electric foot shock every 2 seconds. The grid floors of the non–foot-shock compartments were covered with plastic plates to prevent electric foot shock, and these non–foot-shock compartments were used for the PS and PS+DI rats (Fig. 1).

The stress stimulation within the communication box began 7 days after the electrode installation. Before the day of stress stimulation, the PS, PS+DI, and FS rats