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Psychological stress alters microstructure of the mandibular condyle in rats

Qiang Li [a,1], Min Zhang [a,*,1], Yong-Jin Chen [a,*], Qiang Zhou [a], Ying-jie Wang [a], Jia Liu [b]

[a] Department of General Dentistry & Emergency, School of Stomatology, Fourth Military Medical University, Xi'an, 710032, Shaanxi Province, PR China
[b] Department of Orthodontics, School of Stomatology, Fourth Military Medical University, Xi'an, 710032, Shaanxi Province, PR China

HIGHLIGHTS

- ► Psychological stress induces anxiety in rodents.
- ► Psychological stress induces histomorphological changes in the condylar cartilage.
- ► Psychological stress induces microstructural changes in the condylar bone.
- ► There is a potential link between stress and temporomandibular disorders.

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ABSTRACT

Psychological stress plays an important role in the occurrence and development of temporomandibular joint disorder (TMD). The correlation between psychological factors and TMD has been clinically shown, but the influence of psychological stress on the temporomandibular joint (TMJ) structure still lacks direct evidence. Here, we used communication box to establish the rat model of psychological stress. The stress level of animals was estimated by the elevated plus maze (EPM) test and hormonal assays. The histomorphology and three-dimensional microstructure of the rat condyles were observed by hematoxylin–eosin (HE) staining and Micro-CT, respectively. Compared with control rats, the anxious state of the stressed rats was evidenced by higher plasma adrenocorticotropic hormone (ACTH) and corticosterone (CORT), as well as lower ratios of open arm entries and time and lower time spent in open arms after 1, 2, 3, 4 and 5 week(s) post-exposure to psychological stimuli. HE staining and histomorphometric data analysis showed decreased thicknesses of the central and posterior condylar cartilages in stressed rats at weeks 3, 4 and 5, with the most obvious changes in the posterior part characterized by debonding and thinned fibrous layer, thickened proliferative layer, thinned mature layer and hypertrophic layer. Moreover, Micro-CT scanning revealed local lesion of the subchondral bone in the posterior condylar cartilages of stressed rats at week 5. Our findings indicate that pathologic changes of the histomorphology and three-dimensional microstructure occur in the condyles of stressed rats, hinting us a potential link between psychological factors and the pathogenesis or progression of TMD.

1. Introduction

The temporomandibular joint (TMJ) is a paired diarthrodial joint that connects the lower jaw to the main portion of the skull and permits the lower jaw to move and function. Unlike other joints, the articular portion of the mandibular condyle is composed of fibrocartilage instead of hyaline cartilage [1]. The fibrocartilaginous tissue of mandibular condyles serves as an important load-bearing, shock-absorbing, and lubricating material during physiological activities involving the TMJ [2,3]. The subchondral bone, which is engaged during altered loading [4,5], lies underneath the articular cartilage. The functional integrity of the two structures allows the TMJ to histomorphologically adapt to mechanical loading.

Temporomandibular disorder (TMD), a common disease [6,7], is a collection of conditions affecting the bone and cartilage of TMJ [8]. Numerous studies have illustrated the multifactorial causes of TMD. Among various contributing factors, the psychological factors involved in the etiology of TMD have attracted the attention of researchers in recent years [9–11]. Moreover, clinical studies have shown higher prevalence rates of psychiatric disorders among patients with TMD when compared to healthy people [12], and McNeill [13] included psychological conditions as one of the predisposing and perpetuating factors of TMD. Due to the strong correlation between psychological factors and TMD, researchers have suggested that the presence of psychological distress may influence both TMD onset and progression [9,14]. Nevertheless, until now, proof of the link

* Corresponding authors at: Department of General Dentistry and Emergency, School of Stomatology, Fourth Military Medical University, Changle West Road 145, Xi'an, Shaanxi Province, China. Tel./fax: +86 29 84776488.
E-mail addresses: zhangmin@fmmu.edu.cn (M. Zhang), cyj1229@fmmu.edu.cn (Y.-J. Chen).
[1] These authors contributed equally to the work.

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between psychological factors and TMD has been derived mainly from clinical reports, epidemiological studies, or questionnaire surveys. Direct evidence from animal studies is still lacking.

In our earlier study, we found that psychological stress induced by a communication box can lead to biochemical changes in the orofacial muscles in rats [15]. Episodes of brux-like activity and bruxing behavior in the rodent masseter muscle were also observed with the same paradigm [16]. In addition, it has been reported that the altered activity and modified functioning of masticatory muscles are related to the abnormal physiological structure and functions of the TMJ, such as the histomorphological change and microstructural alteration of subchondral bone beneath the condylar cartilage during pathological processes [17–20]. These facts led us to further explore whether psychological stress can have an adverse effect on the mandibular condyle.

Therefore, the aim of this study was to observe the histomorphology and three-dimensional microarchitecture of mandibular condyles with hematoxylin–eosin (HE) staining and Micro-CT scanning when rats were exposed to psychological stress. We hypothesized that psychological stress induced by a communication box could result in condyle remodeling or even pathological changes in the condylar cartilage and the subchondral bone in the present rat model.

2. Materials and methods

2.1. Animals

A total of 104 male Sprague–Dawley (SD) rats (8 weeks old, weighing 200–220 g) were purchased from the Laboratory Animal Center of the Fourth Military Medical University (Xi'an, China). They were acclimated to laboratory conditions one week before the experiment, with food and water available ad libitum, and then divided into three random groups: the psychological stress group (PS group, 40 rats), the control group (40 rats) and the foot shock group (FS group, 24 rats). The former two groups (PS group and control group) were equally divided into five subgroups according to the observation time points (1, 2, 3, 4 and 5 week(s)), with eight rats in each subgroup. The FS rats that received foot shocks merely served as a source of psychological stress for the PS rats and were not sampled for experimental observation. Eight rats were randomly selected from this group each day to receive a shock in order to minimize anticipation and to prevent adaptation to the stress.

2.2. Psychological stress protocol

Psychological stress was induced by a communication box according to a previously described method [15] (Fig. 1). The electric shocks were generated by a scrambled electrical stimulation generator (Faculty of Biomedical Engineering in the Fourth Military Medical University, Xi'an, China). It output alternating voltage at 48 V by a built-in transformer and produced alternating current at the intensity of 1 mA once every 2 s regardless of the likely varied resistance across rats. We put the PS group rats into the compartments (16 × 16 cm) with a plastic floor to prevent them from receiving electric shocks. The FS group rats received electric foot shock in adjacent compartments with a grid floor, which was composed of stainless steel rods placed 0.3 cm apart. As a result, the PS group rats received psychological stimuli by visual, olfactory and auditory sensation from the FS group rats, which exhibited nociceptive stimulation-evoked responses. Psychological stimuli were given to the animals for 1 h/day (9:00 a.m.–10:00 a.m.). The control group animals were kept in cages 1 h/day without receiving any stress for the duration of the experiment. This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Animal Research Ethics Committee of the Fourth Military Medical University (Xi'an, China).

2.3. Evaluation of anxiety-like behavior level

After measuring the weights of the rats in the PS and control groups at the defined time points, the anxiety-like behavior level of each animal was evaluated by the elevated-plus maze (EPM) test on the plus-shaped maze (RD1208-EP, Shanghai Mobiledatum Corporation, Shanghai, China). In this test, a rat was selected randomly and placed in the center zone, facing the corner between a closed arm and an open arm. Its behavior was recorded using a digital video camera mounted above the maze for 5 min. Before the next rat was introduced, the maze was cleaned with a solution of 20% ethanol and dried to eliminate the odor and trace of the previously tested animal. The behavioral parameters related to anxiety were calculated, which included the ratio of open-arm entries (number of entries into open arms/total number of entries into all arms), the ratio of open-arm time (time spent in open arms/total time in all the arms) and the time spent in the open arms (s) [21,22]. An arm entry was scored when all four paws of animal were placed in an arm.

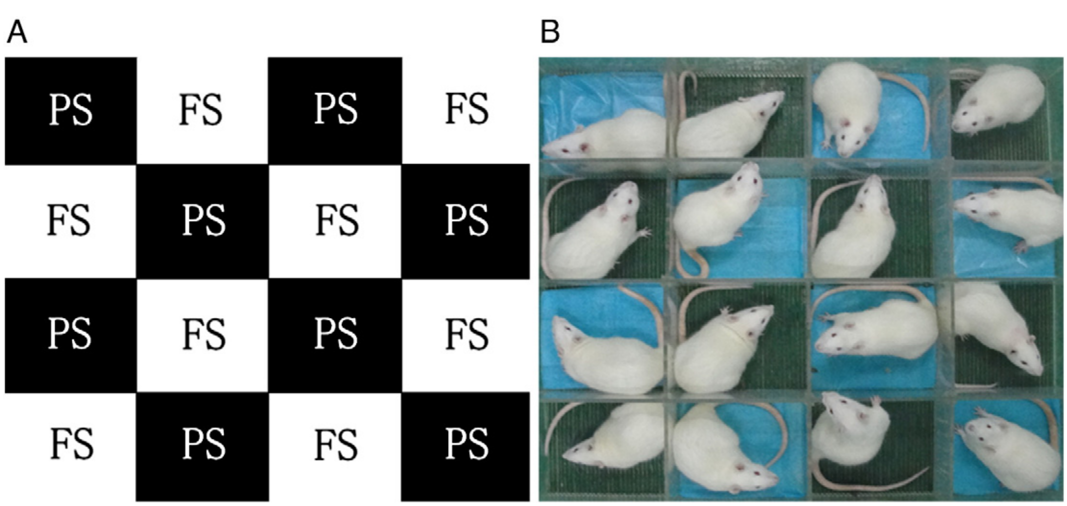

Fig. 1. Schematic diagram of the communication box (A) and operational view of the establishment of animal model of psychological stress (B). The PS group rats were placed individually in the black/blue areas, and received the psychological stress from the FS group rats which were placed individually in the white area. PS, psychological stress group; FS, foot shock group.