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Experimental occlusal disharmony – A promoting factor for anxiety in rats under chronic psychological stress

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ABSTRACT

Background and purpose: Clinically, patients under chronic psychological stress (PS) appear to be more susceptible to occlusal disharmony (OD) compared with those without PS. OD was proved to introduce anxiety-like stress. Therefore, the purpose of the study was to investigate whether OD would affect psychological stress-induced anxiety and its underlying mechanisms.

Methods: Chronic PS was induced by a communication box, and OD was produced by bonding a 0.3 mm-thick crown on the right maxillary first molar of male Sprague-Dawley rats. Sixty-seven rats were randomly divided into 8 groups: (A) chronic PS plus OD group (n = 6); (B) chronic PS plus sham OD group (n = 6); (C) chronic PS only group (n = 6); (D) OD group (n = 6); (E) sham OD group (n = 6); (F) control group (n = 6); (G) naive group (n = 6); (H) foot-shock group (n = 25). Open-field test (OFT) and elevated plus maze test (EPM) were conducted on the 7th, 21th, 35th day to measure the anxiety level of each group except naive and foot-shock group. In addition, corticosterone (CORT) level in serum, 5-hydroxytryptamine (5-HT) and 5-HT_{2A} receptor (5-HT_{2A}R) expressions in prefrontal cortex (PFC), hippocampal CA1 and dentate gyrus (DG) areas were measured on the 35th day to elucidate the mechanism(s) by which the exacerbation occurred.

Results: The significant differences in OFT and EPM tests on day 21 or day 35 between groups ($p < 0.01$) indicated the successful establishment of animal model of PS or OD. And there was a significant increase in CORT concentration in serum ($p < 0.01$), 5-HT expressions in PFC, hippocampal DG areas and 5-HT_{2A}R expressions in PFC, hippocampal CA1 areas ($p < 0.05$) in group A, B, C, D compared with group F. Similar results were also found in group A, B, C, D when compared with group G ($p < 0.05$) except 5-HT expression in DG area in group C and D ($p > 0.05$), together with a gradual decrease in values of all the parameters mentioned above from group A to group G.

Conclusion: The significant changes in exploratory behaviors, serum CORT concentration, 5-HT and 5-HT_{2A}R expressions induced by OD in rats with or without chronic PS, and more obvious alterations in rats with chronic PS, may indicate that OD may be a promoting factor for anxiety through both peripheral and central pathways via the hypothalamus-pituitary-adrenal (HPA) axis and 5-HT system.

1. Introduction

Stress is a physiological and psychological response to environmental changes and noxious stimuli. Chronic stress negatively affects both physical and mental health, which may lead to many illnesses, including anxiety and depressive disorders (Juster et al., 2010; McEwen, 2007; McEwen and Gianaros, 2011; Taylor, 2010). Animals exposed to chronic stress in the lab can generate a variety of adaptive responses, including behavioral, cellular, immune, neuroendocrine alterations (Pacak and Palkovits, 2001). In addition, Baum defined that chronic psychological stress (PS) is a repeated or prolonged negative emotional experience accompanied by behavioral, physiological, and predictable biochemical changes (Baum, 1990).

In clinical dentistry, occlusal disharmony (OD), defined as "a phenomenon in which contacts of opposing occlusal surfaces are not in harmony with other tooth contacts and/or the anatomic and physiologic components of the craniomandibular complex" (The Academy of Prosthodontics, 2005), is common, and can derive from various conditions, including malocclusion, periodontal disease, tooth loss and iatrogenic occlusal alterations such as inadequate occlusal reconstruction and poor restorations. To make the delivery process of definitive restoration more controllable and accurate in prosthetic and restorative dental treatment, multiple apparatus, materials and operating systems for making occlusal record, such as full adjustable mechanical articulators simulating mandibular movements with high precision, addition

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silicone with improved properties, photo occlusion, dental prescale system, 3D virtual articulators with CAD/CAM systems, jaw motion analysis tool, as well as intraoral scanning and digital impression, have been increasingly applied (DeLong et al., 2007; Koralakunte and Aljanakh, 2014; Solaberrieta et al., 2016). However, even with the most accurate technology and materials, minimal alterations in occlusion (such as occlusal surfaces, occlusal curvature and vertical dimension) cannot be completely avoided and occlusion cannot be completely restored compared with the primary occlusal status. Besides, it has been found in our lab that occlusal treatment may become a precipitating factor to trigger stomatognathic problems in susceptible patients when the speed and intensity of occlusal alteration scheme were beyond individual self-regulation range (Jiang et al., 2006). The symptoms caused by occlusal changes might differ in patients with different adaptive capacity. Patients suffering from chronic PS appear to be more susceptible to OD, and might complain about various symptoms, including pain in masticatory muscles and temporomandibular joint, unexplained physical symptoms and mood disorders (such as anxiety and depression) (Vladimir et al., 2012). Psychosocial factors such as increased levels of stress, somatic complaints, and emotional problems seem to play a more prominent role than dental factors in adolescents with temporomandibular disorders (TMD) (List et al., 2001). Moreover, Nieme et al. have indicated that psychological factors appeared significant for the symptom responses to artificial interferences in subjects with an earlier TMD history compared to those without it (Niemi et al., 2006). Therefore, the question arises as to whether OD can exacerbate psychological stress-induced anxiety.

Recent studies have revealed that corticosterone secretion and 5-hydroxytryptamine (5-HT) and 5-HT_{2A} receptor (5-HT_{2A}R) expressions in limbic system, especially prefrontal cortex (PFC), hippocampal CA1 and dentate gyrus (DG) areas, are closely related to mood control(Artigas, 2015; Cox et al., 2011; Duman and Monteggia, 2006; Hammack et al., 2009).

Therefore, the working hypothesis of the present study was that OD may be a promoting factor in exacerbating anxiety in rats under chronic PS. The aim of the present study was to investigate effects of OD on the anxiety level in rats under chronic PS and its underlying mechanisms by evaluation of serum corticosterone concentration and 5-HT and 5-HT_{2A}R expressions in PFC, hippocampal CA1 and DG areas. The results may provide scientific guidance for more rational strategy of occlusal treatment and emotional management.

2. Materials and methods

2.1. Animal preparation

All experimental procedures were approved by the Animal Care and Use Committee of Peking University (Beijing, China) in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (No: LA2013-76). Sixty-seven male Sprague-Dawley rats weighing 160–180 g on arrival were used (Vital River Laboratory Animal Technology Co., Ltd., Beijing, China). All rats were maintained under controlled temperature (22 ± 1 °C) and humidity (50 ± 5%) in a 12 h light/dark cycle (light on 8:00 a.m.) with food and water available ad libitum.

2.2. Animal model of chronic psychological stress (PS)

In this study, PS in rats was induced by responses of electrically shocked rats in the adjacent compartment in a specially designed communication box (Center for Oral Functional Diagnosis, Treatment and Research, Peking University School and Hospital of Stomatology, Beijing, China, illustrated in Fig. 1) as previously described (Li et al., 2013). Briefly, the box consists of 9 compartments (A and B, 16 cm × 16 cm × 16 cm each), separated by transparent plastic boards with several small circular holes (4 holes of 2 cm in diameter in each board). The boards

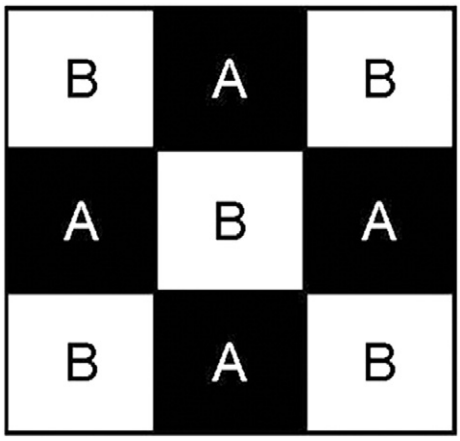

Fig. 1. The schematic diagram of communication box used in present study. The rats in PS associated groups (PS + OD, PS + SOD, PS only group) were individually placed in black areas (compartment A) and received chronic psychological stress caused by electrical shock of rats in FS group, which were placed individually in white areas (compartment B). The grid floors of compartment A were covered by plastic plates and insulated from electricity while those of compartment B were not covered and insulated by plastic plates. The electric foot shocks were delivered to rats in compartment B via stainless wire mesh.

prevented physical contact between animals, but allowed them to receive auditory, visual and olfactory messages from neighboring animals. The bottom of the box was equipped with stainless wire mesh, which was used to induce electric shock when the wires were electrified. The stainless wires were 2 mm in diameter placed at 3 mm intervals. The grid floors of compartment A were covered with transparent plastic boards to prevent animals from electric shock, while grid floors of compartment B were not covered. Animals placed in compartment B received electric foot shocks at 0.5 Hz, 48 V (direct current, DC).

Prior to stress stimulation, all the rats except those in naive group were confined individually in each compartment of the communication box for 30 min per day for 7 days without any electric shocks to get acclimatized to the new surroundings. Then, the rats in the PS and foot-shock (FS) group were respectively put into compartment A and B of communication box at a fixed time (8:00–10:00 a.m.) for 30 min/day from the 8th day to the 35th day. The rats in compartment B (FS group) receiving electric stimuli exhibited nociceptive stimulation-evoked responses such as jumping, shrieks and defecation. The rats confined in compartment A (PS group) did not receive any electrical shock but they were exposed to what the neighboring FS rats were experiencing. The FS rats only served as a resource of chronic psychological stress to the neighboring rats, but did not include in the subsequent experimental evaluations.

2.3. Animal model of occlusal disharmony (OD)

The occlusal disharmony methods have been previously described in several investigations (Cao et al., 2009; Li et al., 2008). Briefly, individual impression trays (Fig. 2) for the rats' dentition with light curing resin denture base were made beforehand. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg), and impressions of the rats' maxillary dentitions were made with silicon rubber (Dental Milestones Guaranteed, Germany), and poured for stone casts. Through a process of trimming work casts, waxing up, investing, casting, fitting on the casts, grinding to certain thickness and polishing, full nickel-chromium crowns or bands for the right maxillary first molars were made. In OD group, the cast metal crown with a thickness of 0.3 mm was bonded to the right maxillary molar of the rats by use of dental resin cement (Panavia F; Kuraray Co., Osaka, Japan) to cover the occlusal, buccal, lingual, and mesial surfaces. Meanwhile, the cast band covering the buccal, lingual, mesial surfaces without changing