Progress in Neuro-Psychopharmacology & Biological Psychiatry 75 (2017) 165–175

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

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

Xuan Tang [a], Jian Li [a,*], Ting Jiang [a,*], Shu-Hui Han [a], Dong-Yuan Yao [b]

[a] Department of Prosthodontics, Peking University School and Hospital of Stomatology, Beijing, People's Republic of China
[b] Jiangxi Mental Hospital and School of Pharmaceutical Science, Nanchang University, Nanchang, Jiangxi, People's Republic of China

ARTICLE INFO

Article history:
Received 22 September 2016
Received in revised form 14 December 2016
Accepted 3 February 2017
Available online 7 February 2017

Keywords:
Psychological stress
Occlusal disharmony
5-HT
5-HT_{2A} receptor
Hypothalamus-pituitary-adrenal axis

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

* Corresponding authors at: Department of Prosthodontics, Peking University School and Hospital of Stomatology, 22 Zhongguancun Nandajie, Haidian District, Beijing 100081, People's Republic of China.
E-mail addresses: pkujianli@126.com (J. Li), jt_ketizu@163.com (T. Jiang).

http://dx.doi.org/10.1016/j.pnpbp.2017.02.006
0278-5846/

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

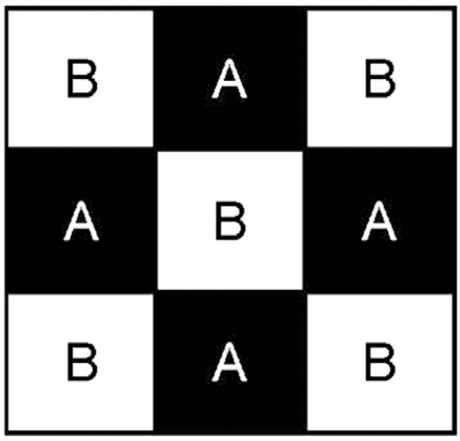

Fig. 1. The schematic diagram of communication box used in present study. The rats in PS associated groups (PS + OD, PS + SOD, PS only group) were individually placed in black areas (compartment A) and received chronic psychological stress caused by electrical shock of rats in FS group, which were placed 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

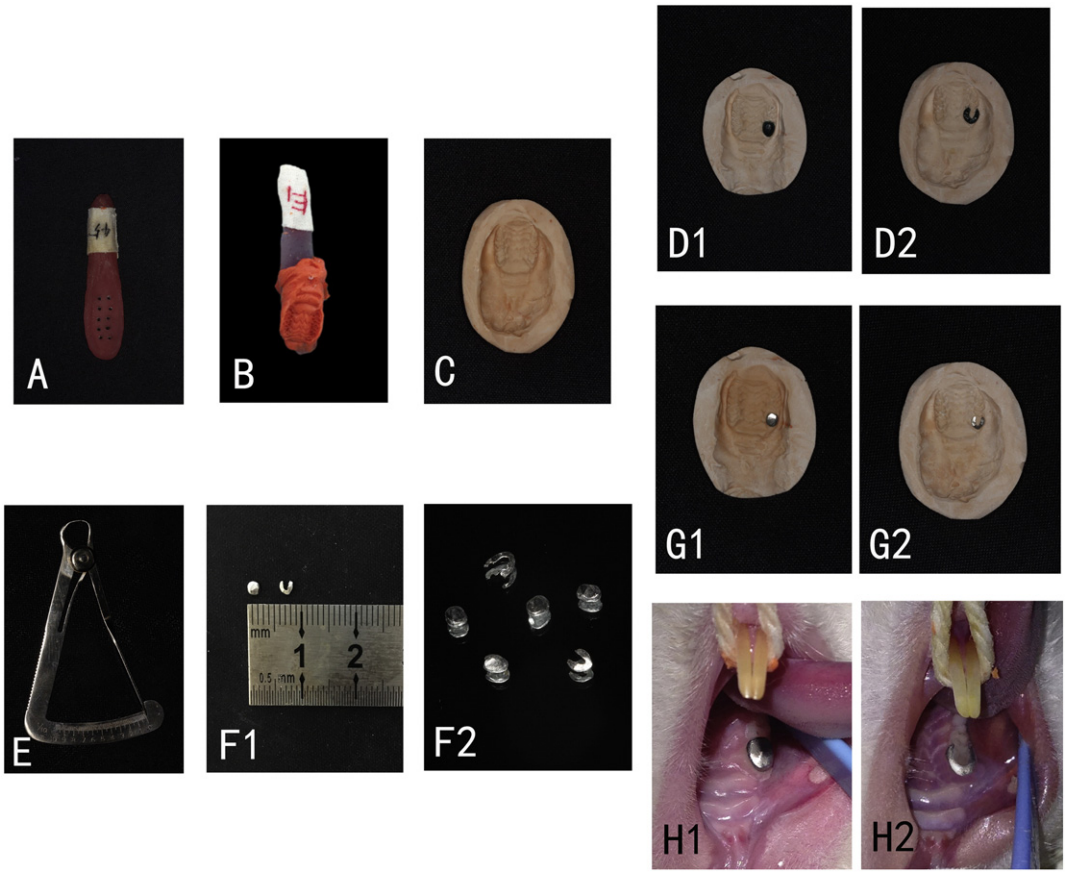

Fig. 2. The schematic diagram of experimental occlusal disharmony. (A) Custom impression tray made with light curing resin denture base. (B) Impression of rat's maxillary dentitions made with silicon rubber. (C) Stone working cast. (D1) Wax pattern of full crown for the right maxillary first molar. (D2) Wax pattern of band for the right maxillary first molar. (E) Calliper used to measure the thickness of occlusal surface in cast metal crown. (F1) The dimensions of cast metal crown or band measured by a steel ruler. (F2) The completed cast crowns and bands. (G1) Cast crown fitting on the working cast. (G2) Cast band fitting on the working cast. (H1) Cast metal crown bonded onto the right maxillary molar. (H2) Cast metal band bonded onto the right maxillary molar. The crown covered the occlusal, buccal, lingual, and mesial surfaces, as shown in D1, G1 and H1. And the band covered the buccal, lingual, mesial surfaces without changing the occlusal surface, as shown in D2, G2 and H2.

the occlusal surface was bonded on the same tooth in sham occlusal disharmony (SOD) group.

2.4. Experimental design and sample collections

The rats were randomly divided into 8 groups: (1) PS + OD group (rats subjected to both chronic psychological stress and occlusal disharmony, n = 6); (2) PS + SOD group (rats subjected to both chronic psychological stress and sham occlusal disharmony, n = 6); (3) PS only group (rats subjected to chronic psychological stress and anesthetized to keep mouth open for 5 min, the same duration as other groups, n = 6); (4) OD group (rats subjected to occlusal disharmony only, n = 6); (5) SOD group (rats subjected to sham occlusal disharmony only, n = 6); (6) CON group (control group, rats anesthetized to keep mouth open for 5 min, n = 6); (7) NAI group (naive group, rats free of any experimental procedures including behavior tests, n = 6); (8) FS group (foot-shock group, rats electrically shocked to induce chronic psychological stress for other rats in neighboring compartments and not included into further study, n = 25). All the rats were housed in the communication box 30 min per day for 7 days to allow them to acclimatize to the box before experiments except NAI group. From the 8th day to the 35th day, all rats except NAI group were put into the box for 30 min per day at 8:00–10:00 a.m. FS rats were electrically shocked to induce chronic psychological stress for rats in PS + OD, PS + SOD, and PS only group. The rats in OD, SOD, and CON group were just confined in the box but not facing psychological stress. Starting from the 22th day, the rats in OD associated groups were subjected to experimental occlusal disharmony for 2 weeks as mentioned above. Behavioral tests (open-field test and elevated plus maze test) were conducted on the 7th, 21th and 35th day to monitor the degree of anxiety in rats. The experimental procedure is illustrated in Fig. 3.

On the 36th day of the study, all the rats were anesthetized in a random order by overdose sodium pentobarbital (50 mg/kg, i.p). Blood from abdominal aorta was quickly collected into glass tubes, centrifuged at 1500*g* for 15 min at 4 °C to extract serum. The serum was stored at

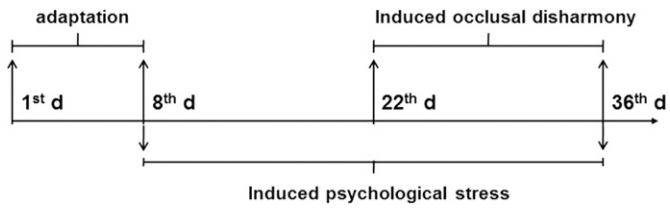

Fig. 3. The schematic diagram of experimental time course. After 7 days adaptation, rats in PS associated groups (PS + OD, PS + SOD, PS only group) were exposed to psychological stress from the 8th day to the 35th day, while rats in the non-PS associated groups (OD, SOD, CON group) were free of chronic PS. On the 22th day, OD with full cast metal crown was induced in both PS + OD and OD group, while SOD with cast band was applied to rats in both PS + SOD and SOD group. Rats in PS only and CON group were just kept mouth open for 5 min to monitor the operation. Rats in NAI group were free of any experimental procedures. At the end of the experiments, all the rats were sacrificed to do subsequent serum assays and immunohistochemical examinations.

−80 °C until needed for assays. At the same time, the brains were dissected on ice and fixed with 4% paraformaldehyde overnight at room temperature for immunohistochemistry.

2.5. Behavioral tests

2.5.1. Open-field test (OFT)

OFT was used to evaluate both locomotor and exploratory activities in all groups except NAI group. The open-field apparatus was a wooden box (100 cm × 100 cm × 50 cm) divided into 25 equal-sized squares by black lines, with a video camera vertically placed 1 m above the test field. The tests were performed on the 7th, 21th and 35th day (8:00–10:00 a.m.). Each rat was gently placed in the center of the arena and allowed to explore freely for 5 min while videotaped to assess horizontal and vertical activities. The apparatus was cleaned with 75% ethanol between each test. The horizontal scores (the number of squares crossed with all four paws) and vertical scores (the frequency of rats rearing up on their hind paws) were counted manually by an observer blinded to the group assignment.

2.5.2. Elevated plus maze (EPM) test

The EPM apparatus was made of black Plexiglas and elevated 70 cm from the floor. It consisted of two open arms (50 cm × 10 cm each) and two equally sized closed arms with 40 cm high walls. The four arms were connected by a central zone (10 cm × 10 cm) in a shape of plus sign. The tests were conducted on the 7th, 21th and 35th day between 10:00 a.m. and 12:00 a.m. under dim light conditions. All rats except those in NAI group were placed individually in the central zone of the maze facing a closed arm and allowed to freely explore the EPM for 5 min. The maze was cleaned with 75% ethanol after each test. The following parameters were recorded by two independent observers who were blinded to the group assignment and sat quietly 2.5 m away from the maze: (1) frequency of open arm entries (OE); (2) frequency of closed arm entries (CE); (3) time spent in open arms (OT); (4) time spent in closed arms (CT). Entry into an arm was defined as the rat placed its four paws in one arm of the maze.

2.6. Serum assay of corticosterone (CORT)

The concentrations of serum CORT were measured with enzyme-linked immunosorbent assay (ELISA) analysis kits (Enzo Life Science Inc., Farmingdale, NY) according to the manufacturer's protocols. The optical density was measured at 405 nm using an ELISA reader (MR-96A; Mindray Co., Shenzhen, China).

2.7. Immunohistochemistry of 5-HT and 5-HT_{2A}R expression

The rat brains were post-fixed with 4% paraformaldehyde for 24 h, and embedded in paraffin after progressive dehydration. Serial coronal sections (4 μ thick) were cut throughout the cerebral cortex and hippocampus using microtome (Leica RM 2125, Germany). The sections were immunostained for 5-HT and 5-HT_{2A}R expression using the Envision two-step method. Briefly, sections were deparaffinized in xylene, followed by heat-mediated antigen retrieval in 10 mM sodium citrate buffer (pH = 6.0) and incubation with the buffer for 30 min at room temperature (RT). Sections were then incubated overnight at 4 °C with primary rabbit anti-5-HT polyclonal antibody (1:100 dilution, ab85596, Abcam, Cambridge, UK) or primary rabbit anti-5-HT_{2A}R polyclonal antibody (1:100 dilution, ab16028, Abcam, Cambridge, UK), subsequently rinsed with 0.1 M phosphate-buffered saline (PBS) four times for 5 min each. Next, secondary antibodies (PV6000, Beijing Zhong Shan-Golden Bridge Biological Technology CO., LTD., Beijing, China) were added, kept there for 30 min at RT and then rinsed with PBS. The immunoactivity was revealed with 3, 3′-diaminobenzidine (DAB, Beijing Zhong Shan-Golden Bridge Biological Technology CO., LTD, Beijing, China) under microscope and stopped by distilled water after 1 min. DAB and counterstaining were performed with hematoxylin. Finally, sections were gradually dehydrated with 80%, 95%, and 100% ethanol twice, 1 min each, cleared with xylene three times for 1 min each, mounted on gelatinized slides and cover slipped.

The PFC, hippocampal CA1 and DG subfields were selected for immunohistochemical assessments by use of a light microscope (Olympus BX53, Tokyo, Japan) and Image-Pro Plus (Version 6.0, Media Cybernetics, Silver Spring, MD, USA). Six random high power fields (400×) in each coronary section were selected and a total of 5 sections per sample were used for quantification in each region. Antibody staining was observed in the cytoplasm. The integral optical density (IOD) of 5-HT and 5-HT_{2A}R immunopositive signals calculated by Image-pro Plus 6.0 was used as quantitative standard of 5-HT and 5-HT_{2A}R expressions, and the mean value of each sample was used for analysis. All the data analyses were performed by a researcher blinded to the group assignment.

2.8. Statistical analysis

Experimental data analyses were performed by one-way analysis of variance (ANOVA) across the groups using SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA). The SNK test was further used for post hoc comparisons. All data were presented as mean ± SD, and $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Behavioral tests

3.1.1. Open-field test

OFT was used to explore motivation and anxiety behavior in a novel environment. No significant differences appeared in horizontal scores (total squares crossed, $F_{(5, 30)} = 0.117$, $p > 0.05$, Fig. 4A) and vertical scores (rearing frequency, $F_{(5, 30)} = 0.102$, $p > 0.05$, Fig. 4B) among groups on day 7. However after two-week psychological stress on day 21, there was a significant reduction in both horizontal scores ($F_{(5, 30)} = 23.856$, $p < 0.01$, Fig. 4A) and vertical scores ($F_{(5, 30)} = 10.551$, $p < 0.01$, Fig. 4B) of rats in PS + OD, PS + SOD, PS only group compared with those in OD, SOD, CON groups. On day 35, after induction of occlusal disharmony, horizontal scores in PS + OD, PS + SOD, PS only group were significantly lower than those in corresponding groups without PS (OD, SOD, CON group) respectively ($F_{(5, 30)} = 11.881$, $p < 0.01$, Fig. 4A), and the scores in PS only, OD, SOD group showed a significant reduction compared with CON group ($p < 0.01$), while there were no significant differences in PS + OD, PS + SOD, PS only group ($p > 0.05$), similar results observed between PS only and OD group ($p > 0.05$). Vertical scores on day 35 in PS + OD, PS + SOD, PS only group were significantly lower than that in OD, SOD, CON group ($F_{(5, 30)} = 5.659$, $p < 0.05$, Fig. 4B), while the values gradually reduced from PS only, PS + SOD to PS + OD group, but no significant difference was observed ($p > 0.05$), similar results were also found in CON, SOD, OD group ($p > 0.05$).

3.1.2. Elevated plus maze test

The anxiety levels of rats were measured by EPM (Fig. 5). On day 7, there were no significant differences among groups in open arm entries (OE, $F_{(5, 30)} = 0.304$, $p > 0.05$), time spent in open arms (OT, $F_{(5, 30)} = 0.245$, $p > 0.05$), closed arm entries (CE, $F_{(5, 30)} = 0.667$, $p > 0.05$) and time spent in closed arms (CT, $F_{(5, 30)} = 0.165$, $p > 0.05$). On day 21, OE, OT, CE in PS + OD, PS + SOD, PS only group were significantly decreased while CT were significantly increased compared with OD, SOD, CON group respectively ($F_{OE\ (5, 30)} = 7.140$, $F_{OT\ (5, 30)} = 88.994$, $F_{CE\ (5, 30)} = 4.124$, $F_{CT\ (5, 30)} = 7.695$, $p < 0.01$), consistent with OFT results. But no significant differences were observed in PS + OD, PS + SOD, PS only group ($p > 0.05$), similar results were also found in OD, SOD, CON group ($p > 0.05$). On day 35, OE, OT, CE in PS + OD, PS + SOD, PS only group were significantly reduced while CT were

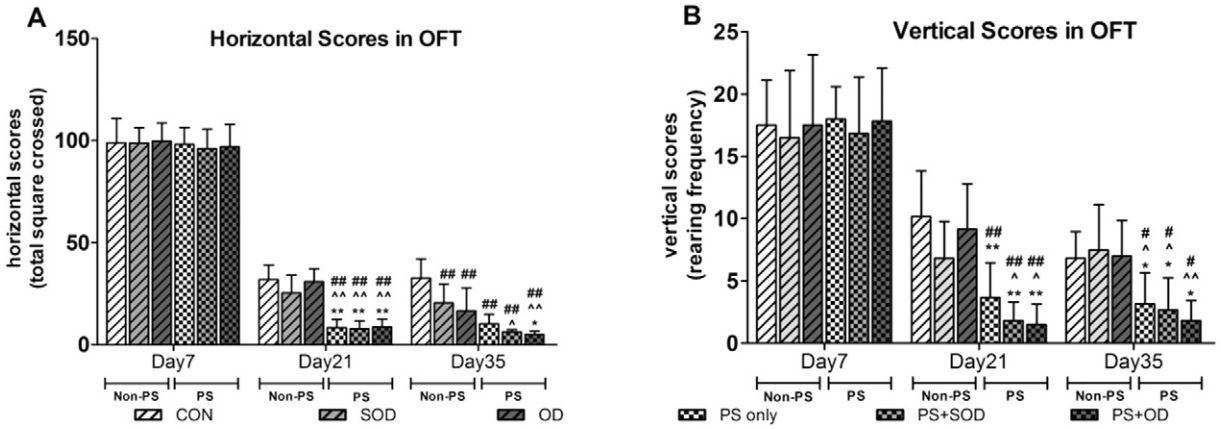

Fig. 4. Open field test on day 7, 21, 35. (A) Horizontal scores, calculated as the total number of squares passed. (B) Vertical scores, calculated as the frequency of rearing. PS associated groups included PS + OD, PS + SOD, PS only group, in which rats experienced chronic psychological stress. OD, SOD and CON group were considered as non-PS associated since rats in these groups did not experience chronic psychological stress. Data are shown as mean ± SD; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with CON group; $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, compared with SOD group; $^{*}p < 0.05$, $^{**}p < 0.01$, compared with OD group.

significantly increased compared with OD, SOD, CON group respectively ($F_{OE\ (5,\ 30)} = 13.027$, $F_{OT\ (5,\ 30)} = 109.747$, $F_{CE\ (5,\ 30)} = 46.630$, $F_{CT\ (5,\ 30)} = 29.563$, $p < 0.01$), most of which were consistent with OFT, except that there was no significant difference of OE in PS + OD, PS + SOD, PS only group compared with OD group ($p > 0.05$). Post hoc comparisons revealed that there were no significant differences of OE, OT, CE, CT in PS + OD, PS + SOD, PS only group on day 35 ($p > 0.05$). While OD group was significantly different compared with CON group in OE, OT,

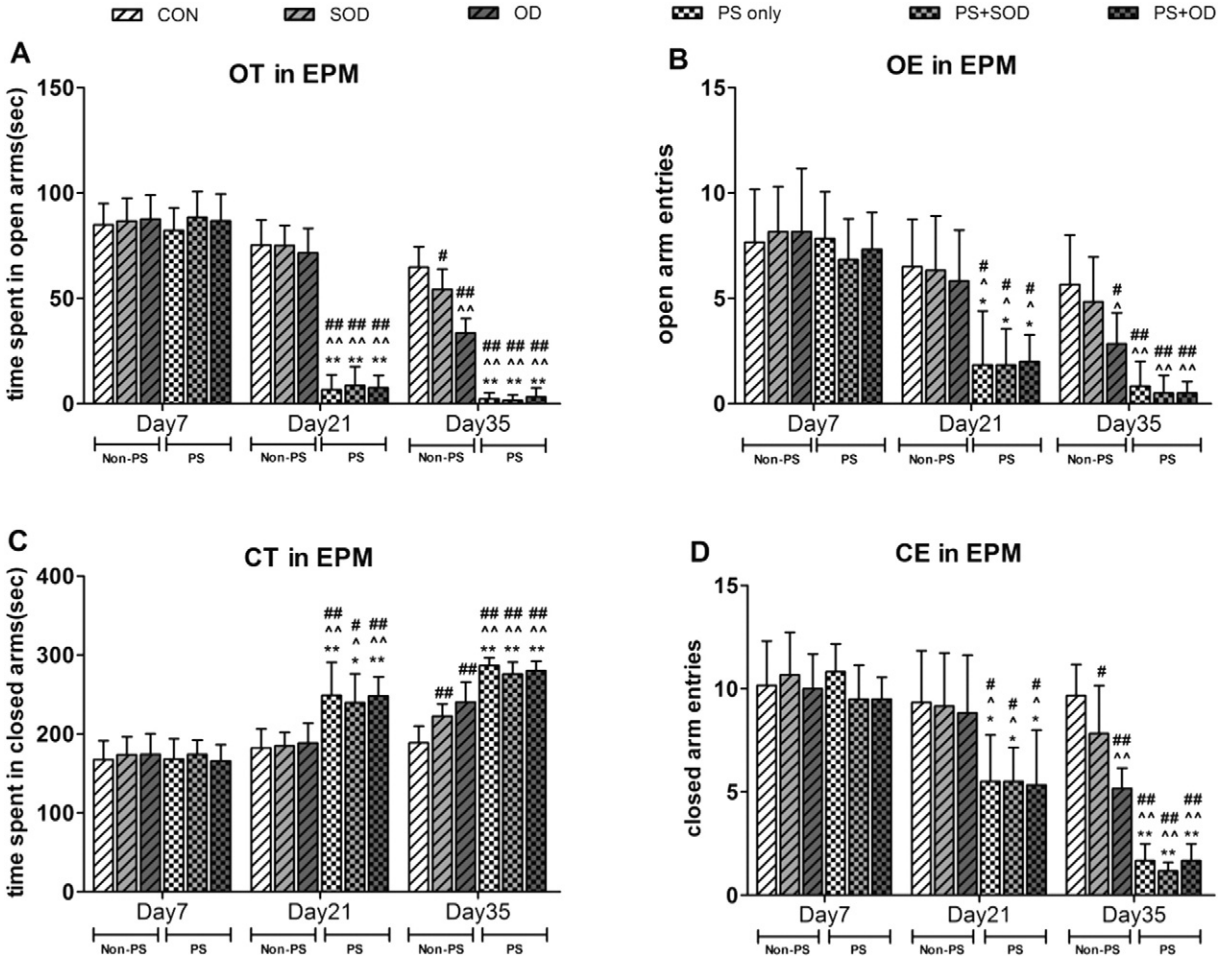

Fig. 5. Elevated plus maze test on day 7, 21, 35. (A) Time spent in open arms in EPM. (B) Number of entries in open arms in EPM. (C) Time spent in closed arms in EPM. (D) Number of entries in closed arms in EPM. PS associated groups include PS + OD, PS + SOD, PS only groups, in which rats experienced chronic psychological stress. OD, SOD, CON groups were considered as non-PS associated since rats in these groups did not experience chronic psychological stress. Data are shown as mean ± SD; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with CON group; $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, compared with SOD group; $^{*}p < 0.05$, $^{**}p < 0.01$, compared with OD group.

CE and CT ($p < 0.05$), and compared with SOD group in OE, OT, CE ($p < 0.05$). Besides, significant differences were also detected in SOD group compared with CON group in OT, CE, CT ($p < 0.05$).

3.2. Concentration of serum corticosterone

The serum CORT concentration was tested to indicate the anxiety level in the experimental rats. As shown in Fig. 6, the serum CORT level in PS associated groups (PS + OD, PS + SOD, PS only group) was significantly increased compared with the rats in corresponding groups without PS (OD, SOD, CON group) respectively ($F_{(6,\ 35)} = 22.021$, $p < 0.01$). In the three PS associated groups, there was a tendency of gradual decrease in serum CORT concentration from PS + OD, PS + SOD to PS only group, but it did not reach statistically significance ($p > 0.05$). And similar tendency of decrease in serum CORT concentration from OD, SOD to CON group (groups without psychological stress) could also be found, while significant reduction in OD group was observed when compared with SOD and CON group ($p < 0.05$). Moreover, all the groups except CON group demonstrated a higher level of serum CORT than NAI group ($p > 0.05$ compared with CON group, $p < 0.05$ compared with SOD group, $p < 0.01$compared with other groups).

3.3. Immunohistochemistry of 5-HT and 5-HT2$_A$R expression

3.3.1. Qualitative assessment

Immunohistochemical tests of 5-HT and 5-HT_{2A}R in the PFC, hippocampal CA1 and DG areas demonstrated strong positive staining in PS + OD group, moderate positive staining in PS + SOD, PS only, OD group, and mild positive or even negative staining in SOD, CON, NAI group(Figs. 7 and 8).

3.3.2. Semi-quantitative assessment

The 5-HT and 5-HT_{2A}R immunoreactivity measured by integral optical density (IOD) was examined in the PFC, hippocampal CA1 and DG areas. Significant differences in 5-HT and 5-HT_{2A}R expressions were observed in the three areas (5-HT: $F_{PFC\ (6,\ 35)} = 17.271$, $F_{CA1\ (6,\ 35)} = 4.469$, $F_{DG\ (6,\ 35)} = 6.582$, $p < 0.01$, Fig. 9A; 5-HT_{2A}R: $F_{PFC\ (6,\ 35)} = 12.900$, $F_{CA1\ (6,\ 35)} = 6.721$, $F_{DG\ (6,\ 35)} = 3.100$, $p < 0.01$, Fig. 9B). Post hoc tests showed that the IOD values of 5-HT and 5-HT_{2A}R immunopositive signals in PS + OD group were higher than that in OD group in all of the three areas, but it failed to reach statistically significance ($p > 0.05$). Meanwhile, significant differences of 5-HT expression in PFC, DG areas and 5-HT_{2A}R expression in PFC area were found in PS + SOD group when compared with SOD group ($p < 0.05$). There were also significant differences of both 5-HT expression in PFC, DG areas and 5-HT_{2A}R expression in PFC, CA1 areas in PS + OD, PS + SOD, PS only, OD group compared with CON group ($p < 0.05$), and similar results were also detected in PS + OD, PS + SOD, PS only, OD group compared with NAI group ($p < 0.05$) except 5-HT expression in DG area in PS only and OD group ($p < 0.05$). But no significant difference was found between PS only and OD group ($p > 0.05$). Besides, the IOD values of both 5-HT and 5-HT_{2A}R expressions in PFC, CA1, DG areas were gradually attenuated from PS + OD, PS + SOD, PS only, OD, SOD, CON to NAI group.

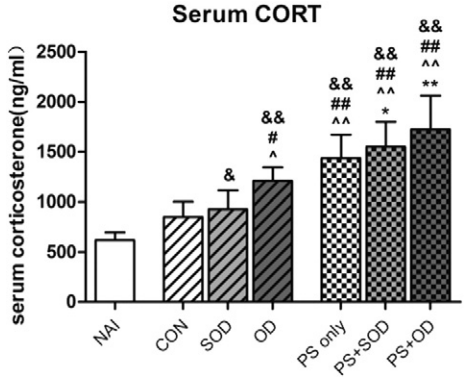

Fig. 6. Serum corticosterone levels on day 36 at the end of the experiment in PS associated groups (PS + OD, PS + SOD, PS only group), Non-PS associated groups (OD, SOD, CON group) and NAI group. Data are shown as mean ± SD; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, compared with NAI group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with CON group; $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, compared with SOD group; $^{*}p < 0.05$, $^{**}p < 0.01$, compared with OD group.

4. Discussion

Mental disorders, especially depression and anxiety, affect over 10% of the world population at any given time (Ferrari et al., 2014), so there is a high possibility for dentists to encounter patients with mental and emotional disorders and it is important to address the issue whether occlusal treatment can affect emotion and anxiety levels in patients. In the present study, we established an animal model to investigate whether the anxiety level in rat undergone chronic psychological stress could be aggravated by dental treatment with and without OD. We have documented that OD could induce the behavioral alterations, elevate the serum CORT secretion in the hypothalamus-pituitary-adrenal (HPA) axis, and increase the expressions of 5-HT and 5-HT_{2A}R in PFC, hippocampal CA1 and DG areas, with more obvious alterations observed in PS rats.

4.1. Successful establishment of the chronic psychologically stressed animal model

The communication box was a relatively well-developed PS inducer. It could induce PS to rodents and effectively separate PS from other interference factors, such as physical stress (Wu et al., 2013). In the present paradigm, communication box was used to induce chronic PS in the rats as they perceived the responses of their neighboring rats exposed to electric foot shock. The successful establishment of the chronic psychologically stressed animal model in PS associated groups was corroborated on day 21 by OFT (Fig. 2) and EPM (Fig. 3), consistent with the results of Yong-jin Chen's research (Chen et al., 2010; Huang et al., 2011). Moreover, the significant differences on day 35 in OFT, EPM tests and serum CORT concentration between groups further indicated that the PS associated groups suffered much more severe anxiety than groups without PS.

4.2. The change of activity of hypothalamus-pituitary-adrenal (HPA) axis induced by occlusal disharmony (OD)

OD is generally considered to cause anxiety-like stress (Budtz-Jorgensen, 1981). Ekuni et al. reported that negative impacts on daily performance attributed to malocclusion may contribute to psychological stress in young Japanese adults (Ekuni et al., 2011a). Various animal models for experimental OD, such as placement of acrylic caps on both lower incisors in Wistar rats (Yoshihara et al., 2001), tooth extraction of upper molar in senescence-accelerated mouse prone-8(SAMP8) mice (Kubo et al., 2007; Onozuka et al., 2001), and cutoff of maxillary molar cusps in Wistar rats (Ekuni et al., 2011b) have displayed an increase in plasma corticosterone levels, an indicator of anxiety (psychological stress). Moreover, previous investigations have indicated that OD activated HPA axis, causing elevated corticosterone levels and possible suppression of hippocampal plasticity in CA1 region (Ekuni et al., 2011b; Kato et al., 2010).

Recent studies have revealed the HPA axis, as a neuroendocrine immune network hub, initiates through the secretion of corticotrophic releasing factor and vasopressin from the hypothalamus, and subsequent release of adrenocorticotrophic hormone (ACTH) from the pituitary then initiates glucocorticoids secretion from the adrenal cortex (cortisol in humans and CORT in rodents) (Huang et al., 2015; Pariante and Lightman, 2008). HPA axis participates in the onset of mood disorders, affects the homeostasis by psychological and physiological reacting to

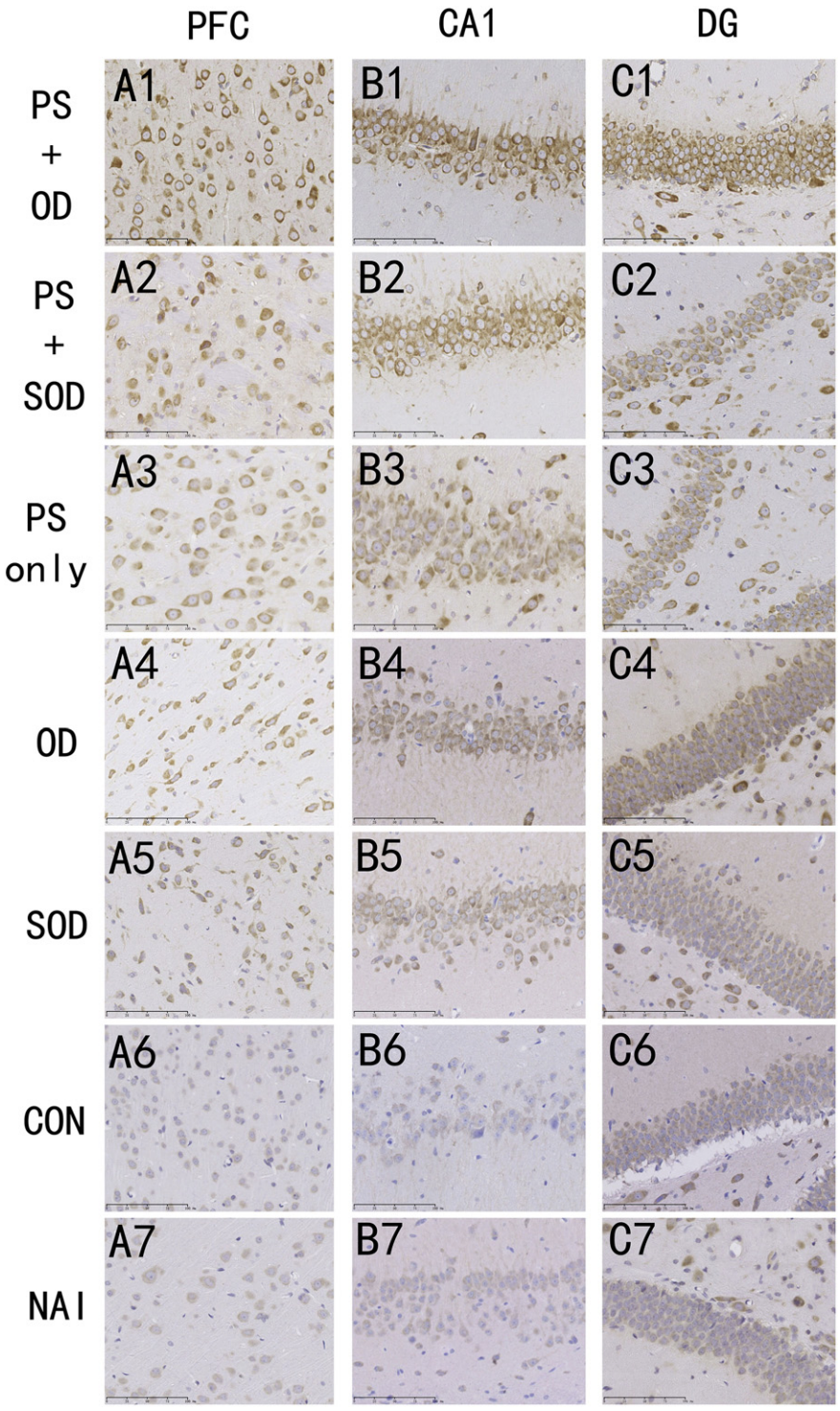

Fig. 7. Immunohistochemical analysis of 5-HT expressions in PFC, hippocampal CA1 and DG subfields on day 35. The number of 5-HT immunopositive cells was increased apparently in PS rats when subjected to OD. Severe vacuolar changes to the cells were observed as the cytoplasm appeared to be more electron-lucent in PS associated groups, especially in PS + OD group. Shown are representative photographs of immunohistochemical staining from PS + OD (A1, B1, C1), PS + SOD (A2, B2, C2), PS only (A3, B3, C3), OD (A4, B4, C4), SOD (A5, B5, C5), CON (A6, B6, C6), and NAI (A7, B7, C7) group.

environmental changes and regulating emotions in human (Zhao, 2000). F. Holsboer reported that stress caused pathological alterations in the stress-responsive HPA axis to causality of depression (Holsboer, 2000). Elevated corticosterone levels were proved to be associated with stress activation of the HPA axis (Cox et al., 2011). The activity of HPA axis in the present study was increased by OD in PS + OD and OD group, as shown by elevated serum CORT compared to the corresponding groups without OD (PS + SOD, SOD, PS only, CON group), implying that our animal model was potent enough to induce anxiety, especially when psychologically stressed rats borne with OD.

4.3. The main regions in brain affected by stress

Emotion processing in hippocampus and amygdala, as well as the connected regulation of PFC, would be the most affected regions in brain by chronic stress (Bremner, 2006; Perkins et al., 2013). The

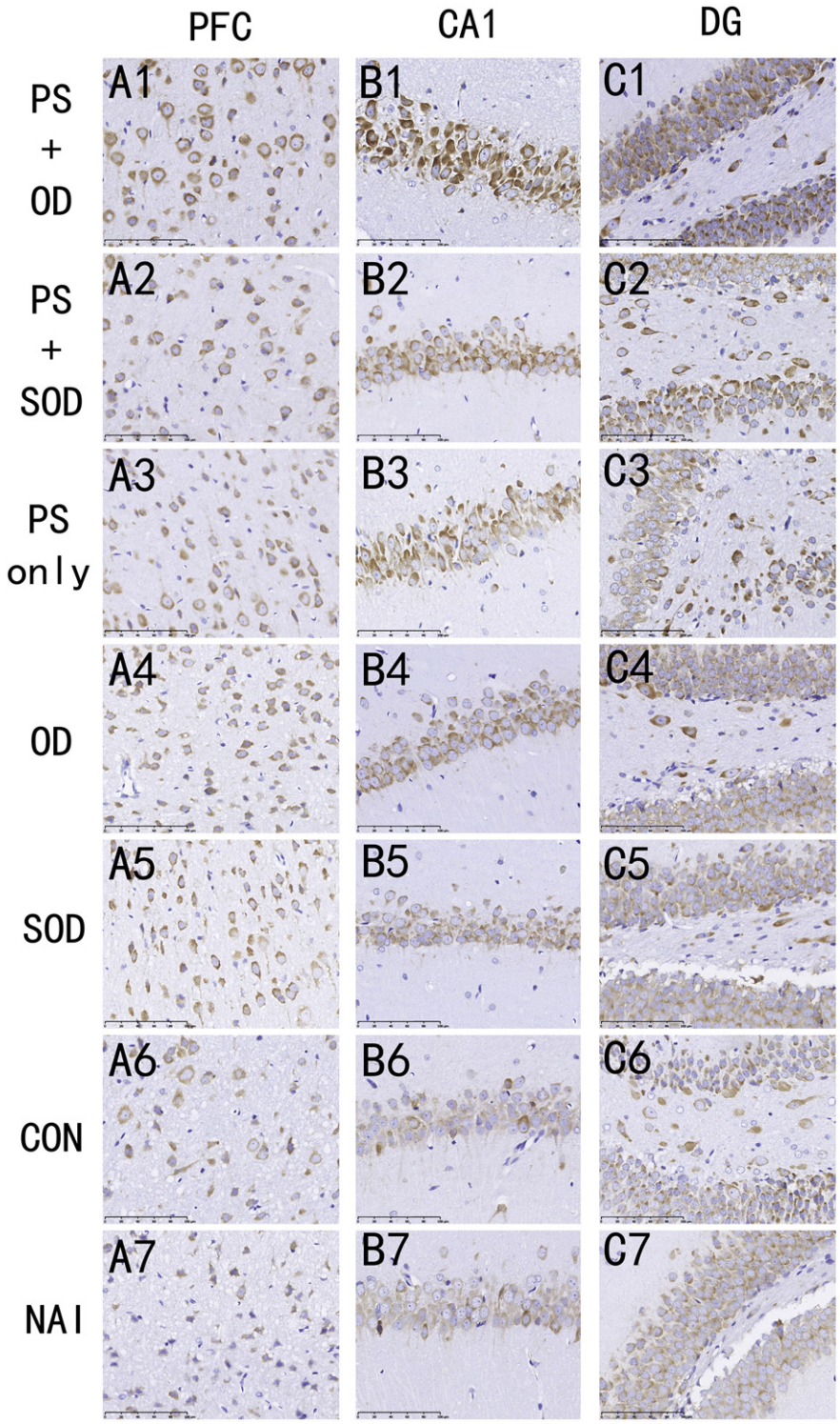

Fig. 8. Immunohistochemical analysis of 5-HT_{2A}R expressions in PFC, hippocampal CA1 and DG subfields on day 35. The number of 5-HT_{2A}R immunopositive cells was increased apparently in PS rats when subjected to OD. Shown are representative photographs of immunohistochemical staining from PS + OD (A1, B1, C1), PS + SOD (A2, B2, C2), PS only (A3, B3, C3), OD (A4, B4, C4), SOD (A5, B5, C5), CON (A6, B6, C6), and NAI (A7, B7, C7) group.

stress-sensitive hippocampus, involved in anxiety, depression, regulation of the HPA axis, learning, recognition and other autonomic functions, has received significant attention recently, with multiple studies based on PS (D'Sa and Duman, 2002; Warner-Schmidt and Duman, 2006). The seahorse-shaped structure, with high levels of receptors for glucocorticoid and stress hormones, is one of the most vulnerable brain structures to stress conditions. Studies have revealed that stress mainly has effects on the CA1, CA3 pyramidal cell layers and DG granule cell layer among the major subfields of hippocampus (Kim and Diamond, 2002; Swaab et al., 2005). The hippocampus also has important connections with the PFC and amygdala that may further underlie the affective disorders (Duman and Monteggia, 2006). The monoaminergic systems (particularly 5-HT and norepinephrine) on which the antidepressant drugs target, are tightly controlled by PFC, a cortical region where both metabolic and morphological abnormalities have been reported in depression (Artigas, 2015). In present study, PFC,

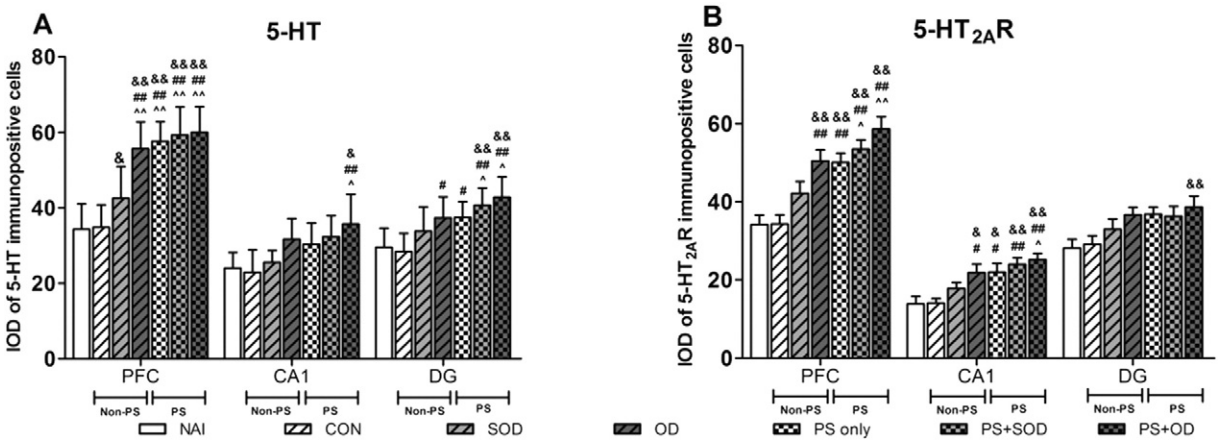

Fig. 9. The expression of 5-HT and 5-HT_{2A}R in PFC, hippocampal CA1 and DG subfields on day 35 at the end of the experiment examined by immunohistochemical staining in PS associated groups (PS + OD, PS + SOD, PS only group), non-PS associated groups (OD, SOD, CON group) and NAI group. (A) The integral optical density (IOD) of 5-HT immunopositive cells. (B) IOD of 5-HT_{2A}R immunopositive cells. Data are shown as mean ± SD; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, compared with NAI group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with CON group; $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, compared with SOD group.

hippocampal CA1 and DG areas were observed changing synchronously to PS and OD, which also indicated the connected neuroplasticity among these areas.

4.4. The regulation mechanism of 5-HT system in mood disorders

The 5-HT system plays an important role in the etiology of neuropsychiatric disorders such as depression and anxiety (Parks et al., 1998). The synthesis, release, reuptake and metabolic disorders of 5-HT and its receptors are closely linked with affective disorders, social relationship deterioration and lack of pleasure (Yang, 2000). 5-HT, as a key neurotransmitter in mood control, is the major target of drugs for anxiety disorders, with substantial evidence showing that changes in 5-HT functioning can modulate fear and anxiety-like behavior in humans and animals (Bandelow et al., 2008; Hammack et al., 2009). Selective activation of the inhibitory response mediated by 5-HT_{1A} receptors to 5-HT reduces anxiety-like behavior, and the activation of the excitatory response mediated by 5-$HT_{2A/2C/4/6/7}$ receptors to 5-HT may be anxiogenic (Hammack et al., 2009). The 5-HT receptor subtypes, particularly 5-HT_{2A}R, have been postulated to play a significant role in depression and anxiety (Pandey et al., 2002). 5-HT_{2A} receptors are densely found in the PFC, hippocampus and amygdale, which have close connections with anxiety, cognition, depression and schizophrenia (Papp et al., 1994). Studies have indicated that depression is due to pathologically up-regulating 5-HT_{2A}R function in brain marginal zone (Huang et al., 2015). And numerous antidepressants and antipsychotic agents binding with relatively high affinity at 5-HT_{2A}R (Artigas, 2015), further confirms the impact of 5-HT_{2A}R on emotions. Y. Kurhe et al. found that selective 5-HT_{2A}R antagonist successfully reduced chronic unpredictable mild stress-induced depression (Kurhe et al., 2015), thus reflecting another line of evidence demonstrating the effect of 5-HT_{2A}R. Besides, previous study showed that the blockade of 5-HT_{2A}R enhanced the 5-HT_{1A} receptor-mediated neurotransmission, which links to the antidepressant efficacy (Artigas, 2015), and 5-HT_{2A}R could also regulate the dopaminergic and noradrenergic neurotransmission, both relevant to mood regulation, motivation and cognition (Song et al., 2015). Stress could augment the levels of dopamine, serotonin and their metabolites in certain brain regions (Blanchard et al., 1991; Munck et al., 1984). Consistent with the previous studies, significant increase of 5-HT and 5-HT_{2A}R expressions in PFC area was observed in PS + OD, PS + SOD, PS only, OD group compared with CON and NAI group in the present study, which indicated that the stress of PS or OD could affect mood regulation via serotonin system. However, in the current study, 5-HT_{2A}R expressions were significantly increased in CA1 area in PS + OD, PS + SOD, PS only, OD group compared with CON and NAI group, while significant increase was observed only in PS + OD group in DG area. We inferred that the different results of 5-HT_{2A}R expression in hippocampal CA1 and DG areas may be due to the different quantity, distribution and affinity of 5-HT_{2A}R in CA1 pyramidal cells and DG granule cells (Jakab and Goldman-Rakic, 1998; Katsuyuki et al., 1997; Kim and Diamond, 2002; Swaab et al., 2005). On the contrary, 5-HT expressions were significantly increased in DG area in PS + OD, PS + SOD, PS only, OD group compared with CON group, while significant increase was observed only in PS + OD group in CA1 area. Previous study indicated that 5-HT firing activity was generally increased by stress (Ace, 2005). As the 5-HT release increases, the elevated 5-HT activates 5-HT_{1A} receptors with an inhibitory role on 5-HT, and 5-$HT_{2A/2C/4/6/7}$ receptors with an excitatory role, in order to maintain the regular discharge rate of 5-HT neurons (Berumen et al., 2012; Hammack et al., 2009; Whitaker-Azmitia, 2005). Therefore, due to the combined action of both inhibitory and excitatory receptors, no significant increase of 5-HT expression in OD, PS only, PS + SOD group in CA1 area was detected in order to keep homeostasis, while, the homeostasis in the PS + OD group may be interrupted, resulting in increased 5-HT. Besides, it has been reported that the excess 5-HT caused a desensitization of 5-HT_{1A} receptors with a lesser inhibition, which may lead to a facilitation of 5-HT signaling (Corradetti et al., 1998). So we speculated that the excitatory effects of 5-$HT_{2A/2C/4/6/7}$ receptors in DG area would be stronger than the inhibitory effects of 5-HT_{1A} receptors, thus increased the 5-HT release in most groups in DG area. Moreover, the higher 5-HT and 5-HT_{2A}R expressions in PFC, hippocampal CA1 and DG areas in PS + OD group may indicate the promoting effect on the exacerbation of mood regulation induced by OD in chronic psychologically stressed rats.

Additionally, cumulative evidence has indicated that the activation of the HPA axis changes the function of the 5-HT system. Chronic administration of CORT or ACTH increased the number of wet-dog shakes induced by DOI (a 5-HT_{2A} receptor agonist), which may shed light on the mechanism governing the 5-HT_{2A}R's up-regulation (Kawakami et al., 2005; Kitamura et al., 2002; Katsuyuki et al., 1997). The increased serum CORT concentrations in PS associated groups were paralleled by the increased 5-HT expressions in PFC, hippocampal DG areas and 5-HT_{2A}R expressions in PFC, hippocampal CA1 areas in our study, may also demonstrate stress activation of HPA axis on the 5-HT and 5-HT_{2A}R regulation.

4.5. Possible transmission mechanism of stress stimulus

Stress affects both central and peripheral nervous system. It activates the central control stations located in the hypothalamus which includes the corticotrophic releasing hormone (CRH) and arginine-

vasopressin(AVP) neurons of the paraventricular nuclei (PVN), and the locus ceruleus (LC) regulating the central sympathetic system (norepinephrinesystem), and causes an increase in secretion of glucocorticoids, catecholamines, opiates and other neuromodulators, which are intimately associated with limbic structures, including the hippocampus, amygdala and medial prefrontal cortex (Kim and Diamond, 2002). When exposed to stress, the brain influences all body organs via the HPA axis together with the efferent sympathetic system, which represent the effector limbs (Constantine and Chrousos, 2002).

As the final common pathway for the mood disorder symptomatology, the HPA axis is driven by stress with the elevated secretion of CRH and AVP in the hypothalamus, which then triggers ACTH release from pituitary, and subsequently stimulates glucocorticoid (CORT or cortisol) release from the adrenals. Elevated corticosterone may change the brain serotonergic regulation, via directly affecting presynaptic 5-HT_{1A} receptors or postsynaptic 5-HT_{2A}, 5-HT_{1A} receptors (Katsuyuki et al., 1997). In turn, 5-HT plays a stimulatory role of the ACTH and CORT secretion in HPA axis by ways of $5HT_{2A}$ and perhaps $5HT_{1A}$ receptors (Parks et al., 1998). The 5-HT system, innervating forebrain stress integrative structures, is postulated to be involved in mediating stress effects on the hippocampus, PFC, amygdala, and hypothalamus (Herman et al., 2003). To restore the homeostasis, the hippocampus and the PFC inhibit the HPA axis, while the amygdala activates the axis. The hippocampus, with high density of glucocorticoid receptors, can directly terminate the stress response via glucocorticoid-mediated negative feedback, inhibits the CRH and AVP mRNA expressions in parvocellular PVN neurons directly, and subsequently suppresses the descending pathway of ACTH and CORT secretion (Herman and Cullinan, 1997). In addition, the viewpoint has been increasingly accepted that the full response of stress-induced effects on the hippocampus requires cooperation of the hippocampus and amygdala. The amygdala, connected to the hippocampus directly by amygdalo-hippocampal bundles arising from the basolateral amygdale and terminating in CA1 area or indirectly via the entorhinal cortex, influences the hippocampal function in concert with neuromodulators (including CORT, 5-HT, CRF and opiates)(Kim and Diamond, 2002; Pikkarainen et al., 1999). Like hippocampus, several studies also indicated that the PFC selectively modulated stress-induced HPA axis activity with innervations to several PVN-projecting regions (Herman et al., 2003; Swaab et al., 2005). Exposure to stress triggers the central and peripheral circuits illustrated above to restore homeostasis. However, if the sustained stress is beyond the allostatic load, the hyperfunction of HPA axis could cause deleterious effects on the hippocampus, involving neurogenesis of the DG granule neurons and synaptogenesis of the CA1 pyramidal neurons, and could also inhibit PFC metabolism (McEwen, 2001). As a result, the interactions between the HPA axis and the limbic structures are out of control, which are of crucial importance in the pathogenesis of mood disorders.

4.6. Relations between chronic psychological stress and orofacial pain

The view that chronic stress can induce anxiety or depression has been widely verified by preclinical tests. As a factor that can precipitate mood disorders, chronic stress can alter brain structure and activity, produce neuronal atrophy and impair neurogenesis in subregions of hippocampus, including disruption of HPA axis and modulation of 5-HT metabolism (McEwen, 2001). Repeated or prolonged stress in animals can also decrease nociceptive thresholds, thus induces hyperalgesia. Chronic repeated psychological stress-induced anxiety is directly related to masticatory muscle pain with increased mechanical sensitivity (Huang et al., 2011). While OD, as a chronic stressor, is believed to be directly related to masticatory muscle pain (Cao et al., 2009), activation of the HPA axis and deterioration of hippocampal plasticity (Kato et al., 2010). The clinical connection between pain and affective disorders has long been recognized (Burke et al., 2010), and the increased 5-HT metabolism in brain structures may be involved in pain perception and mood disorders (Burke et al., 2010; Nowak et al., 2013). In the present study, as for the increased concentration of serum CORT and expressions of 5-HT, 5-HT_{2A}R in brain in OD group compared to CON group, we considered OD, as a stressor, working through HPA axis and 5-HT system, may have an influence on mood disorders, and we speculated that OD may also affect pain perception via 5-HT systems. Further study would be applied to investigate whether or to what extent OD could induce the changes in pain threshold of masticatory muscles in subjects under chronic psychological stress.

4.7. Limitations

Due to the limitation coming from animal experiments, we could not obtain the serum CORT concentration and 5-HT, 5-HT_{2A}R expression of the same animal on day 21 compared with that on day 35 to analyze the time-line development of peripheral and central neuroplasticity induced by occlusal disharmony in chronic psychologically stressed rats.

5. Conclusion

In conclusion, an animal model of PS combined with OD to mimic clinical situations of occlusal alteration in patients with psychological problems was successfully established in the study. OD, as well as PS, could induce anxiety in rats through both peripheral and central pathways via the HPA axis and 5-HT system. Compared with non-PS rats, OD may be a promoting factor in aggravating the anxiety level in psychologically stressed rats, indicated by more obvious alterations in stress associated behaviors and expressions of transmitter and associated receptors in the brain. Therefore, it is necessary to be more cautious when giving occlusal treatment to patients under psychological stress. A thorough evaluation of the patient, including dental, psychological and comprehensive medical assessments, is needed before dental intervention is involved, especially when complicated dental treatment such as full mouth rehabilitation and irreversible occlusion adjustment is planned to be offered. The more rational strategy is that emotional management should be prior to occlusal treatment. And the speed and intensity of occlusal treatment scheme on patients with emotion problems should be lower than those without.

Author's contributions

X.T., S.H.H. and J.L. carried out the animal and laboratory experiments. X.T. wrote a draft of the manuscript. J.L. and T.J. contributed to the design of the study and critical review of the manuscript. D.Y.Y. helped with the interpretation of findings and the final revision of the manuscript.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (NO. 81100771). The authors are deeply thankful to Bin Yang and Wei-Hong Cong for their technological assistance in immunohistochemical study.

References

Ace, L., 2005. Stress, corticotropin-releasing factor and serotonergic neurotransmission. Handbook of Stress and the Brain. 1, pp. 503–524.
Artigas, F., 2015. Developments in the field of antidepressants, where do we go now? Eur. Neuropsychopharmacol. 25, 657–670.
Bandelow, B., Zohar, J., Hollander, E., Kasper, S., Moller, H.J., Zohar, J., Hollander, E., Kasper, S., Moller, H.J., Bandelow, B., et al., 2008. World federation of societies of biological psychiatry (WFSBP) guidelines for the pharmacological treatment of anxiety, obsessive-compulsive and post-traumatic stress disorders - first revision. World J. Biol. Psychiatry 9, 248–312.
Baum, A., 1990. Stress, intrusive imagery, and chronic distress. Health Psychol. 9, 653–675.
Berumen, L.C., Rodríguez, A., Miledi, R., García-Alcocer, G., 2012. Serotonin receptors in hippocampus. Sci. World J. 5, 1–15.

Blanchard, D.C., Cholvanich, P., Blanchard, R.J., Clow, D.W., Hammer Jr., R.P., Rowlett, J.K., Bardo, M.T., 1991. Serotonin, but not dopamine, metabolites are increased in selected brain regions of subordinate male rats in a colony environment. Brain Res. 568, 61–66.
Bremner, J.D., 2006. The relationship between cognitive and brain changes in posttraumatic stress disorder. Ann. N. Y. Acad. Sci. 1071, 80–86.
Budtz-Jorgensen, E., 1981. Occlusal dysfunction and stress. An experimental study in macaque monkeys. J. Oral Rehabil. 8, 1–9.
Burke, N.N., Hayes, E., Calpin, P., Kerr, D.M., Moriarty, O., Finn, D.P., Roche, M., 2010. Enhanced nociceptive responding in two rat models of depression is associated with alterations in monoamine levels in discrete brain regions. Neuroscience 171, 1300–1313.
Cao, Y., Xie, Q.F., Li, K., Light, A.R., Fu, K.Y., 2009. Experimental occlusal interference induces long-term masticatory muscle hyperalgesia in rats. Pain 144, 287–293.
Chen, Y.J., Huang, F., Zhang, M., Shang, H.Y., 2010. Psychological stress alters ultrastructure and energy metabolism of masticatory muscle in rats. J. Biomed. Biotechnol. 2010, 302693.
Constantine, T., Chrousos, G.P., 2002. Hypothalamic-pituitary-adrenal axis,neuroendocrine factors and stress. J. Psychosom. Res. 53, 865–871.
Corradetti, R., Laaris, N., Hanoun, N., et al., 1998. Antagonist properties of (-)-pindolol and WAY 100635 at somatodendritic and postsynaptic 5-HT1A receptors in the rat brain. Br. J. Pharmacol. 3, 449–462.
Cox, B.M., Alsawah, F., McNeill, P.C., Galloway, M.P., Perrine, S.A., 2011. Neurochemical, hormonal, and behavioral effects of chronic unpredictable stress in the rat. Behav. Brain Res. 220, 106–111.
DeLong, R., Knorr, S., Anderson, G.C., Hodges, J., Pintado, M.R., 2007. Accuracy of contacts calculated from 3D images of occlusal surfaces. J. Dent. 35, 528–534.
D'Sa, C., Duman, R.S., 2002. Antidepressants and neuroplasticity. Bipolar Disord. 4, 183–194.
Duman, R.S., Monteggia, L.M., 2006. A neurotrophic model for stress-related mood disorders. Biol. Psychiatry 59, 1116–1127.
Ekuni, D., Furuta, M., Irie, K., Azuma, T., Tomofuji, T., Murakami, T., Yamashiro, T., Ogura, T., Morita, M., 2011a. Relationship between impacts attributed to malocclusion and psychological stress in young japanese adults. Eur. J. Orthod. 33, 558–563.
Ekuni, D., Tomofuji, T., Irie, K., Azuma, T., Endo, Y., Kasuyama, K., Morita, M., 2011b. Occlusal disharmony increases amyloid-beta in the rat hippocampus. Neruomol. Med. 13, 197–203.
Ferrari, A.J., Norman, R.E., Freedman, G., Baxter, A.J., Pirkis, J.E., Harris, M.G., Page, A., Carnahan, E., Degenhardt, L., Vos, T., et al., 2014. The burden attributable to mental and substance use disorders as risk factors for suicide: findings from the global burden of disease study 2010. PLoS One 9, e91936.
Hammack, S.E., Guo, J.D., Hazra, R., Dabrowska, J., Myers, K.M., Rainnie, D.G., 2009. The response of neurons in the bed nucleus of the stria terminalis to serotonin: implications for anxiety. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 33, 1309–1320.
Herman, J.P., Cullinan, W.E., 1997. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci. 20, 78–84.
Herman, J.P., Figueiredo, H., Mueller, N.K., Ulrich-Lai, Y., Ostrander, M.M., et al., 2003. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Front. Neuroendocrinol. 24, 151–180.
Holsboer, F., 2000. The stress hormone system is back on the map. Curr. Psychiatry Rep. 2, 454–456.
Huang, F., Zhang, M., Chen, Y.J., Li, Q., Wu, A.Z., 2011. Psychological stress induces temporary masticatory muscle mechanical sensitivity in rats. J. Biomed. Biotechnol. 2011, 720603.
Huang, H., Zhao, J., Jiang, L., Xie, Y., Xia, Y., Lv, R., Dong, L., 2015. Paeoniflorin improves menopause depression in ovariectomized rats under chronic unpredictable mild stress. Int. J. Clin. Exp. Med. 8, 5103–5111.
Jakab, R.L., Goldman-Rakic, P.S., 1998. 5-Hydroxytryptamine2A serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. Proc. Natl. Acad. Sci. U. S. A. 95, 735–740.
Jiang, T., Li, J., Jin, Z., Wang, Y.W., Feng, H.L., Ishikawa, T., 2006. Comparison of atypical orofacial pain and temporomandibular disorders synovitis pain processing in the human brain using functional magnetic resonance imaging. Zhonghua Kou Qiang Yi Xue Za Zhi 41, 670–673.
Juster, R.P., McEwen, B.S., Lupien, S.J., 2010. Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci. Biobehav. Rev. 35, 2–16.
Kato, K., Ono, Y., Kubo, K.Y., Sasaguri, K., Watanabe, K., Onozuka, M., Sato, S., 2010. Occlusal disharmony suppresses long-term potentiation in the rat hippocampal CA1 region. J. Stomat. Occ. Med. 3, 71–75.
Katsuyuki, T., Tadashi, N., Yoshihisa, K., Yamawaki, S., 1997. Effects of corticosterone on 5-HT1A and 5-HT2A receptor binding and on the receptor-mediated behavioral responses of rats. Eur. J. Pharmacol. 333, 123–128.
Kawakami, Y., Kitamura, Y., Araki, H., Kitagawa, K., Suemaru, K., Shibata, K., Gomita, Y., 2005. Effects of monoamine reuptake inhibitors on wet-dog shakes mediated by 5-HT2A receptors in ACTH-treated rats. Pharmacol. Biochem. Behav. 81, 65–70.
Kim, J.J., Diamond, D.M., 2002. The stressed hippocampus, synaptic plasticity and lost memories. Nat. Rev. Neurosci. 3, 453–462.
Kitamura, Y., Araki, H., Suemaru, K., Gomita, Y., 2002. Effects of imipramine and lithium on wet-dog shakes mediated by the 5-HT2A receptor in ACTH-treated rats. Pharmacol. Biochem. Behav. 72, 397–402.
Koralakunte, P.R., Aljanakh, M., 2014. The role of virtual articulator in prosthetic and restorative dentistry. J. Clin. Diagn. Res. 8, 25–28.
Kubo, K.Y., Yamada, Y., Iinuma, M., Iwaku, F., Tamura, Y., Watanabe, K., Nakamura, H., Onozuka, M., 2007. Occlusal disharmony induces spatial memory impairment and hippocampal neuron degeneration via stress in SAMP8 mice. Neurosci. Lett. 414, 188–191.
Kurhe, Y., Mahesh, R., Devadoss, T., 2015. QCM-4, a 5-HT(3) receptor antagonist ameliorates plasma hpa axis hyperactivity, leptin resistance and brain oxidative stress in depression and anxiety-like behavior in obese mice. Biochem. Biophys. Res. Commun. 456, 74–79.
Li, J., Jiang, T., Feng, H., Wang, K., Zhang, Z., Ishikawa, T., 2008. The electromyographic activity of masseter and anterior temporalis during orofacial symptoms induced by experimental occlusal highspot. J. Oral Rehabil. 35, 79–87.
Li, Q., Zhang, M., Chen, Y.J., Zhou, Q., Wang, Y.J., Liu, J., 2013. Psychological stress alters microstructure of the mandibular condyle in rats. Physiol. Behav. 110–111, 129–139.
List, T., Wahlund, K., Larsson, B., 2001. Psychosocial functioning and dental factors in adolescents with temporomandibular disorders: a case-control study. J. Orofac. Pain 15, 218–227.
McEwen, B.S., 2001. Plasticity of the hippocampus:adaptation to chronic stress and allostatic load. Ann. N. Y. Acad. Sci. 933, 265–277.
McEwen, B.S., 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol. Rev. 87, 873–904.
McEwen, B.S., Gianaros, P.J., 2011. Stress- and allostasis-induced brain plasticity. Annu. Rev. Med. 62, 431–445.
Munck, A., Guyre, P.M., Holbrook, N.J., 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5, 25–44.
Niemi, P.M., Le Bell, Y., Kylmala, M., Jamsa, T., Alanen, P., 2006. Psychological factors and responses to artificial interferences in subjects with and without a history of temporomandibular disorders. Acta Odontol. Scand. 64, 300–305.
Nowak, P., Kowalinska-Kania, M., Nowak, D., Kostrzewa, R.M., Malinowska-Borowska, J., 2013. Antinociceptive effects of H3 (R-methylhistamine) and GABA(B) (baclofen)-receptor ligands in an orofacial model of pain in rats. Neurotox. Res. 24, 258–264.
Onozuka, M., Watanabe, K., Fujita, M., Tonosaki, K., Saito, S., 2001. Evidence for involvement of glucocorticoid response in the hippocampal changes in aged molarless SAMP8 mice. Behav. Brain Res. 131, 125–129.
Pacak, K., Palkovits, M., 2001. Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. Endocr. Rev. 22, 502–548.
Pandey, G.N., Dwivedi, Y., Rizavi, H.S., Ren, X., Pandey, S.C., Pesold, C., et al., 2002. Higher expression of serotonin 5-HT(2A) receptors in the postmortem brains of teenage suicide victims. Am. J. Psychiatry 159, 419–429.
Papp, M., Klimek, V., Willner, P., 1994. Effects of imipramine on serotonergic and beta-adrenergic receptor binding in a realistic animal model of depression. Psychopharmacology 114, 309–314.
Pariante, C.M., Lightman, S.L., 2008. The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 31, 464–468.
Parks, C.L., Robinson, P.S., Sibille, E., Shenk, T., Toth, M., 1998. Increased anxiety of mice lacking the serotonin1A receptor. Proc. Natl. Acad. Sci. U. S. A. 95, 10734–10739.
Perkins, S.C., Finegood, E.D., Swain, J.E., 2013. Poverty and language development: roles of parenting and stress. Innov. Clin. Neurosci. 10, 10–19.
Pikkarainen, M., Ronkko, S., Savander, V., Insausti, R., Pitkanen, A., 1999. Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in rat. J. Comp. Neurol. 403, 229–260.
Solaberrieta, E., Garmendia, A., Brizuela, A., Otegi, J.R., Pradies, G., Szentpetery, A., 2016. Intraoral digital impressions for virtual occlusal records: section quantity and dimensions. Biomed. Res. Int. 2016, 7173824.
Song, J., Hou, X., Hu, X., Lu, C., Liu, C., Wang, J., Liu, W., Teng, L., Wang, D., 2015. Not only serotonergic system, but also dopaminergic system involved in albiflorin against chronic unpredictable mild stress-induced depression-like behavior in rats. Chem. Biol. Interact. 242, 211–217.
Swaab, D.F., Bao, A.M., Lucassen, P.J., 2005. The stress system in the human brain in depression and neurodegeneration. Ageing Res. Rev. 4, 141–194.
Taylor, S.E., 2010. Mechanisms linking early life stress to adult health outcomes. Proc. Natl. Acad. Sci. U. S. A. 107, 8507–8512.
The Academy of Prosthodontics, 2005. The glossary of prosthodontic terms. J. Prosthet. Dent. 94, 10–92.
Vladimir, L.S., Leesa, M., Eric, L.S., 2012. Pain and persistent occlusal awareness: what should dentists do? J. Am. Dent. Assoc. 143, 989–991.
Warner-Schmidt, J.L., Duman, R.S., 2006. Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. Hippocampus 16, 239–249.
Whitaker-Azmitia, P.M., 2005. Behavioral and cellular consequences of increasing serotonergic activity during brain development: a role in autism? Int. J. Dev. Neurosci. 23, 75–83.
Wu, G., Chen, L., Fei, H., Su, Y., Zhu, G., Chen, Y., 2013. Psychological stress may contribute to temporomandibular joint disorder in rats. J. Surg. Res. 183, 223–229.
Yang, Q., 2000. Central control of stress reaction in hypothalamic-pituitary-ovarian axis. Sheng Li Ke Xue Jin Zhan 31, 222–224.
Yoshihara, T., Matsumoto, Y., Ogura, T., 2001. Occlusal disharmony affects plasma corticosterone and hypothalamic noradrenaline release in rats. J. Dent. Res. 80, 2089–2092.
Zhao, W.L., 2000. Recent researches on neurotransmitters and neuroendocrine system of affective disorder. Guangxi Med. J. 22, 534–536.