BMC Oral Health

RESEARCH

Open Access

The association between traumatic temporomandibular joint bony ankylosis and depressive disorder in growing rats

Tiange Deng[1†], Yang Xue[1†], Yiming Wang[1†], Xiang Guo[1], Zhen Ma[1], Hongzhi Zhou[1], Zaiyong Yang[2], Zhengwu Peng[3], Xiangxiang Hu[4], Ning Li[5], Kaijin Hu[6*] and Lei Wang[7*]

Abstract

Background The psychological symptoms of temporomandibular joint (TMJ) ankylosis were similar to that of depressive disorder, but there were no relevant evidences to confirm that the humans or animals with TMJ ankylosis had depressive disorder. The aim of this study was to investigate the association between TMJ ankylosis and depressive disorder in the rat model.

Methods Thirty 3-week-old male Sprague–Dawley (SD) rats were used in this study. The damage of TMJ complexes and narrowed joint space were performed in the unilateral TMJ of test group to induce TMJ bony ankylosis (experimental side). The other TMJ of test group underwent a sham operation (sham side). The TMJs of control group did not undergo any operations. At 8 weeks postoperatively, behavioral tests, body weight, passive maximum mouth opening (PMMO), and TMJ morphological features were evaluated, and the hippocampuses were analyzed using western blotting and immunocytochemistry. The data was compared between the test group and control group by independent t-test, between the experimental side and sham side by paired t-test. The correlations between PMMO/area of bony fusion and duration of immobility, sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and the number of BrdU-positive cell were evaluated using linear regression analysis. The level of significance was 0.05.

Results In the test group, the traumatic TMJ complexes with narrowed joint space developed TMJ bony ankylosis, the area of bony mass of experimental side (21.26 mm^2) was larger than that of sham side (1.73 mm^2) ($p < 0.001$). There were significant difference with the sucrose preference (test group: 0.36, control group: 0.76, $p < 0.001$), duration of immobility (test group: 127.36 s, control group: 59.41 s, $p < 0.001$), body weight (test group: 156.70 g, control group: 270.06 g, $p < 0.001$), PMMO (test group: 9.98 mm, control group: 28.79 mm, $p < 0.001$), CB1 receptor protein (test group: 41.00%, control group: 86.69%, $p < 0.001$), mean optical density of CB1 receptor protein (test group: 29.60 a.u., control group: 54.69 a.u., $p < 0.001$), and the number of BrdU-positive cell between the test group and control group (test group: 2133.71, control group: 4301.95, $p < 0.001$). PMMO was negatively correlated with the duration

†Tiange Deng, Yang Xue and Yiming Wang contributed equally to this work.

†Lei Wang and Kaijin Hu contributed equally to this work.

*Correspondence:
Kaijin Hu
hukaijin2015@126.com
Lei Wang
wangleixsk@sina.cn
Full list of author information is available at the end of the article

of immobility ($r = 0.953$, $p < 0.001$), while the area of bony fusion was positively correlated (r = 0.961, $p < 0.001$). PMMO was positively correlated with the sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and the number of BrdU-positive cell ($r = 0.955, 0.955, 0.976, 0.958$, $p < 0.001$, all), while the area of bony fusion was negatively correlated ($r = 0.970, 0.981, 0.971, 0.958$, $p < 0.001$, all).

Conclusions The present study verified that depressive disorder was found in the rat model of traumatic TMJ bony ankylosis. The severity of TMJ bony ankylosis correlated with the severity of depressive disorder.

Keywords TMJ ankylosis, Maximum mouth opening, Area of bony fusion, Depressive disorder, Sucrose preference, Duration of immobility, CB1 receptor protein, BrdU-positive cell

Background

The temporomandibular joint (TMJ) ankylosis is characterized by abnormal bony or fibrous adhesions after trauma or disease, this condition has significant effects on quality of life, with symptoms including masticatory difficulty, pain, and so on [1–3], which further leads to adverse mental and psychological disorders [1, 3, 4]. Depressive disorder is one of the most common mental and psychological diseases, which is characterized by consistent depressed mood, appetite pattern change, loss of pleasure, behavior motor retardation, and feelings of worthlessness [5–8].

The TMJ ankylosis is a particular temporomandibular disorder and osteoarthritis [4, 9–12]. Previous studies reported that chronic pain and mouth opening limitation frequently coexists with depressive disorder in the temporomandibular disorder and osteoarthritis [13–20]. However, the specific relationships between the physical function of TMJ disorder or osteoarthritis and the severity of depressive disorder are not investigated. Marie et al. reported that the pain and mouth opening limitation could lead to a complete ankylosis of the TMJ, and the patients could felt depressed because of TMJ problems [11]. Vinay et al. reported that the improved physical signs of patients with TMJ ankylosis were accompanied with the improved psychosocial symptoms, such as breathing difficulty, sleep disorders, and the problems of psycho-social wellness [21]. Our previous study showed that traumatic TMJ bony ankylosis could lead to the limitation of mouth opening and the decrease of body weight, and there were the negative correlation between maximum mouth opening and the severity of TMJ bony ankylosis [1, 2]. Taken together, the abovementioned data suggest that physical signs of patients or animals with TMJ ankylosis coexists with psychological symptoms of depressive disorder. Although the psychological signs and symptoms of TMJ ankylosis was similar to that of depressive disorder in the previuous study, there was no conclusive evidence to confirm that the patients or animals with TMJ ankylosis had depressive disorder, and the association between TMJ ankylosis and depressive disorder remain unclear.

The aim of this study was to (1) verify the occurrence of TMJ bony ankylosis and depressive disorder in a rat model; (2) investigate the association between the severity of depressive disorder and the severity of TMJ bony ankylosis, with developing appropriate treatment approaches for traumatic patients that can prevent TMJ bony ankylosis and depressive disorder in the future.

Methods

Animal care

Sprague–Dawley male rats at 3 weeks old (weight 60–80 g) were provided by the Laboratory Animal Center of the Fourth Military Medical University (FMMU, Xi'an, China). Animals were housed to habituate for 1 week under standard laboratory conditions and given free access to tap water and standard rodent diet. This study was approved by the animal ethics committee (Approval ID 2020–0950).

Study design

The SD rats were divided into test group ($n = 20$) and control group ($n = 10$). The sample size was referred to our previous study [3], based on the mean sucrose preference test (SPT) of the pre-test with 12 SD rats (test group 0.63 ± 0.08 and control group 0.76 ± 0.09), the shedding rate of the study sample was usually 20%, and the minimum sample size was 30 (PASS 11.0, Test for Two Means: Power = 0.93, $\alpha = 0.05$, $R = 2.0$). The rats in the control group did not receive any surgical treatment. The rats in the test group were subjected to unilateral TMJ complexes trauma to establish TMJ bony ankylosis (experimental side). In the experimental side, a 1 cm long curved preauricular incision was made and the flap was lifted to expose the TMJ complex. The capsule was exposed by blunt dissection. The condyle was isolated with a periosteal elevator, and the articular disc was exposed by the separation of the lateral attachment of the disc through the joint space. The anterior and posterior attachments of the disc were then cut off and the articular disc was removed. The joint space was exposed by horizontal blunt dissection, the fibrocartilage on the condyle was

removed using a Gracey scaler and grid grooves were carved using an apex elevator on the surface of the glenoid fossa. The joint space was then narrowed, the capsule was sutured, and the wound was closed in layers.

The other TMJ complexes of the test group underwent a sham operation (sham side) whereby a curved preauricular incision was made and the TMJ complex was exposed, then the wound was closed in layers. After surgery, all rats were administered the penicillins (2 mg/100 g; X–Y Biotechnology) and pentazocine (0.1 mg/100 g; X–Y Biotechnology) for 7 days.

At 8 weeks after surgery, the behavioral tests were performed before general anesthesia, the body weight, passive maximum mouth opening (PMMO), and TMJ morphological features using micro-CT were measured and performed under general anesthesia, and then all the rats were euthanized by intravenous injection of a lethal dose of pentobarbital sodium, the details of procedures were described previously [3]. The samples of TMJ complexes were harvested from the euthanized animals for morphological and radiological evaluation. The hippocampuses were rapidly separated and frozen in liquid nitrogen until western blotting and immunocytochemistry test were performed.

Micro-CT

All the rats were scanned under general anesthesia in vivo by micro-CT (the parameters: 80kV tube voltage, 500μA anode current, 10μm revolution rate, Siemens AG)) at 8 weeks postoperatively. Subsequently, The trabecular micro-architecture of TMJ complexes was evaluated using the built-in software of the micro-CT device (the Inveon Research Workplace, Siemens AG). Morphological features, including the shape and erosion patterns of the glenoid fossa and condylar surfaces, as well as the degree of calcification of the bony fusion areas were evaluated (bony fusion was determined and defined by areas of calcification). The maximum horizontal area of the bony fusion were each measured three times by a technician who was not otherwise involved in this study. The evaluators were blind. The mean value of each measurement was calculated for analysis [2].

Behavioral tests

Forced swimming test (FST)

The forced swimming test is one of the most commonly used animal models for assessing depressant-like behaviour [22]. At 8 weeks postoperatively, the rats were forced to swim individually in a testing plastic cylinder (40 cm in depth × 20 cm in diameter) filled with water (24 ± 1 °C) for 6 min and the immobile time (floating in the water with an upright position and stopped struggling) was recorded only in the last 5 min, FST involves in scoring of active (swimming and climbing) or passive (immobility) behaviours, their behavior was videotaped for later analysis, and the duration of immobility was scored and recorded by blinded observers. The blinded observers were not involved in this study and were not aware of group allocation and treatment conditions. The duration of immobility indicating a state of helplessness was determined. Following the swimming session, the rats were dried with towels and kept warm under a lamp in their home cages [23, 24].

Sucrose preference test (SPT)

The sucrose preference test was conducted to determine the anhedonia. Prior to the test, rats were deprived of food and water for 20 h at 8 weeks postoperatively. During the test, animals were allowed to consume water and 1% sucrose solution freely for 1 h. After 1 h, sucrose and water consumption (ml) was measured. The evaluators were blind, were not involved in this study and were not aware of group allocation and treatment conditions. The sucrose preference was calculated according to the following ratio: sucrose preference (%) = sucrose consumption (g)/(sucrose consumption (g) + water consumption (g) [24, 25].

Western blotting (WB)

To assess the effects of TMJ bony ankylosis on cannabinoid type I receptor (CB1) protein expression, which was determined by WB. The hippocampuses were rapidly frozen in liquid nitrogen and carried with routine procedures. WB analysis was performed using the standard protocol of molecular cloning (Edition II). The protein concentrations was visualized and analyzed using the BCA Protein Assay Kit (Invitrogen; Thermo Fisher Scientific, Inc.). The samples were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a poly vinylidene fluoride (PVDF) membrane. The membrane was incubated with following primary antibodies: rabbit anti-CB1 receptor (1:200, Abcam, Cambridge, UK) antibody and anti-β-actin antibodies (1:5000, Abcam, Cambridge, UK), after blocking with 5% non-fat dried milk. Electrophoresis was carried out by SDS-PAGE with 10% polyacrylamide gels. Subsequently, The membranes were then washed thrice in the trisbuffered saline and incubated with secondary antibodies goat anti-mouse IgG (1:10,000, Abcam, Cambridge, UK) for 1 h at room temperature. After washing thrice (8 min each) in the trisbuffered saline, the immunoreactive proteins were visualized and analyzed using the chemiluminescence detection kit (34,077, SuperSignal West Pico Chemiluminescent Substrate, Thermo, USA) [24]. The evaluators were blind, were not involved

in this study and were not aware of group allocation and treatment conditions.

Immunocytochemistry

To label proliferating cells, the rats were intraperitoneal injected with 5-Bromo- 2'-deoxyuridine (bromodeoxyuridine (BrdU)) (100 mg/kg in saline; Sigma, St. Louis, MO, USA) for three days and sacrificed on the fourth day for immunostaining. The hippocampus was cut in the coronal plane into 20 mm thick sections with a cryostat and mounted on gelatinized slides [26, 27]. To investigate BrdU-positive cell phenotype, All the sections were incubated in 2 N HCl for 30 min and then rinsed in 0.1 M borate buffer (pH 8.5) for 10 min. After washing in PBS, sections were incubated with anti-mouse BrdU (B8434, 1:500, SigmaeAldrich) overnight. Which were later followed by the incubation with an anti-mouse Alexa Fluor 594-conjugated secondary antibody. All the sections were observed under a fluorescence microscope. Every sixth section throughout the entire hippocampal formation was processed for BrdU immunofluorescent analysis, at least 10 sections were examined per animal, and the number was multiplied by six to estimate the total number of BrdU-labeled cells per dentate gyrus [26]. To analyze CB1 receptor distribution, all the sections were incubated overnight on a shaker in rabbit anti-CB1 polyclonal antibody. All the slides were imaged with a fluorescent Olympus BX- 51 microscope (Tokyo, Japan). All the images were analyzed with Image-Pro plus software (Media Cybernetics, Rockville, MD, USA) [24, 25]. The evaluators were blind, were not involved in this study and were not aware of group allocation and treatment conditions.

Statistical analysis

The SPSS 25.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The data was presented as mean ± SD. Data normality was tested using the Kolmogorov–Smirnov test. The data was compared between the test group and control group by independent t-test. The data was compared between the experimental side and sham side in the test group by paired t-test. The correlations between PMMO/area of bony fusion and duration of immobility, sucrose preference, CB1 receptor protein, mean optical density, and the number of BrdU-positive cell were evaluated using linear regression analysis. A p-value of < 0.05 was considered statistically significant.

Results

The measurement of body weight and PMMO and findings of gross observation

All the rats could tolerate the compound trauma in the test group, and no postoperative wound infection occurred. The average body weight in the test group and control group was 156.70 ± 19.81 g, 270.06 ± 14.21 g, respectively, there was significant difference between the test group and control group at 8 weeks post-surgery ($p < 0.001$). The PMMO was also measured and recorded at 8 weeks postoperatively (yellow line) (Fig. 1A). The average PMMO in the test group and control group was 9.98 ± 2.83 mm, 28.79 ± 2.14 mm, respectively, there was significant difference between the test group and control group ($p < 0.001$) (Fig. 2A). On the experimental side, a raised hard mass of the TMJ external appearance was observed with the naked eye and felt by palpation (yellow arrow) (Fig. 1B). TMJ bony ankylosis and raised new grown bone mass on the TMJ complex surfaces were observed (yellow arrow) (Fig. 1C). Furthermore, the joint space disappeared on the experimental side, and bony fusion area of TMJ complex was observed in the joint space (yellow arrow) (Fig. 1D). Bony fusion area of TMJ complex was absent on the sham side (black arrow) (Fig. 1E).

The findings of micro-CT

The 3D reconstruction of micro-CT revealed more new bone formation on the experimental side but not on the sham side, TMJ bony ankylosis occurred in the test group at 8 weeks after surgery (red arrow) (Fig. 3A). In the axial CT of experimental side, the joints showed calcified callus formation in the joint space and a narrowed joint space (red arrow) (Fig. 3B). In the coronal CT of experimental side, the joints showed some calcified bone bridge formation through the joint space with nearly disappearance of the joint space) (red arrow) (Fig. 3C). In the sagittal CT of experimental side, the joints showed the irregular formation of new bone, which protruded into the joint space (red arrow) (Fig. 3D). The areas of bony mass (bony fusion area of experimental side and condylar area of sham side) were analysed and calculated in the test group (Fig. 3E). The bony fusion area of experimental side was 21.26 ± 3.57 mm^2. The condylar area of sham side was 1.73 ± 0.53 mm^2. The area of bony mass was significantly different between the experimental side and sham side ($p < 0.001$) (Fig. 2B).

The findings of forced swimming test and sucrose preference test

The duration of immobility in the test group and control group was 127.36 ± 10.32 s, 59.41 ± 9.23 s, respectively, there was significant difference between the test group and control group in the forced swimming test at 8 weeks post-surgery ($p < 0.001$) (Fig. 4A). In addition, the sucrose preference in the test group and control group was 0.36 ± 0.14, 0.76 ± 0.17, respectively, there was significant difference between the test group and control group in

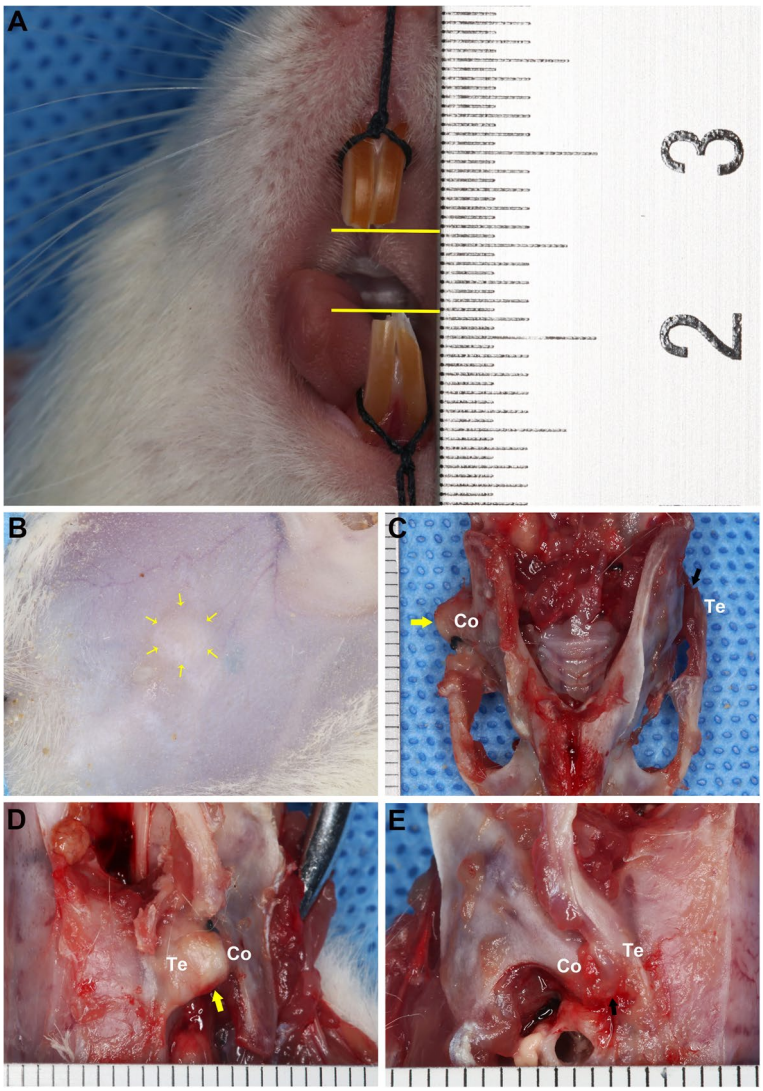

Fig. 1 Measurement of passive maximum mouth opening and gross observation of the TMJ complexes in the test group at 8 weeks postoperatively. **A** Passive maximum mouth opening, which was defined as the distance between two yellow lines; **B** The raised fusion mass of TMJ bony ankylosis was seen in the front of the tragus (yellow small arrow); **C** Bony fusion mass of condyle and temporal bone was seen on the experimental side (yellow arrow) but not on the sham side (black arrow); **D** Disappeared joint space between condyle and glenoid fossa was seen on the experimental side (yellow arrow); **E** Joint space between condyle and glenoid fossa was obviously seen on the sham side (black arrow); Co: condylar process; Te: temporal bone

the sucrose preference test at 8 weeks post-surgery ($p < 0.001$)(Fig. 4B).

The hippocampal expression of the CB1 receptor protein

The CB1 receptor localization and expression was examined by double immunofluorescence staining for red CB1 receptor and green NeuN (neuronal marker). The CB1 receptor localization and expression was observed by double immunofluorescence staining in the control group (Fig. 5A-C). The CB1 receptor localization and expression was observed by double immunofluorescence staining in the test group (Fig. 5D-F). The CB1 receptor expression in the test group and control group was 41.00 ± 3.16%, 86.69 ± 7.36%, respectively, there was significant difference between the test group and control group in the WB test at 8 weeks post-surgery ($p < 0.001$) (Fig. 5G). Furthermore, The mean optical density of CB1 receptor protein expression in the test group and control group was 29.60 ± 2.55 a.u., 54.69 ± 3.00 a.u., respectively, there was significant difference between the test group and control group at 8 weeks post-surgery ($p < 0.001$) (Fig. 5H).

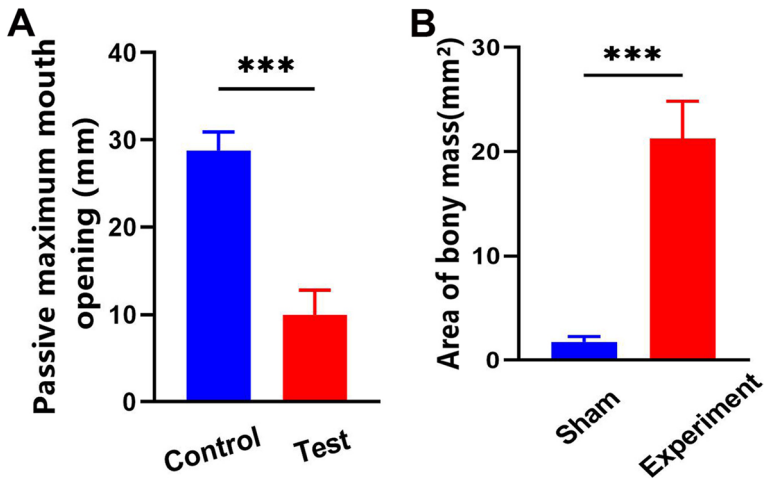

Fig. 2 Comparison of passive maximum mouth opening between two group and bony mass area between two sides at 8 weeks postoperatively. **A** Comparison of passive maximum mouth opening between control group and test group; **B** Comparison of bony mass area between sham side and experimental side in the test group; Data represent mean ± SD; *,**,***: $p < 0.05, 0.01, 0.001$

The hippocampal expression of the BrdU-positive cells

The BrdU-positive cell localization and expression was examined by double immunofluorescence staining for red BrdU-positive cell and green NeuN (neuronal marker) at 8 weeks post-surgery. The BrdU-positive cell localization and expression was observed by double immunofluorescence staining in the control group (Fig. 6A-C). The BrdU-positive cell localization and expression was observed by double immunofluorescence staining in the test group (Fig. 6D-F). The number of BrdU-positive cells in the dentate gyrus was counted using the modified stereology protocol. The BrdU-positive cell number of the dentate gyrus in the test group and control group was 2133.71 ± 139.40, 4301.95 ± 138.01, respectively, there was significant difference between the test group and control group ($p < 0.001$) (Fig. 6G).

The association between the indicators of TMJ bony ankylosis and the indicators of depressive disorder

Figure 7 presents the associations between the PMMO and the duration of immobility, sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and the number of BrdU-positive cell. At 8 weeks post-surgery, the PMMO was negatively correlated with the duration of immobility. However, the PMMO was positively correlated with the sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and the number of BrdU-positive cell. The results of linear regression analysis revealed the PMMO to be an independent variable affecting the duration of immobility, sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and the number of BrdU-positive cell. The linear regression equations for these relationships were shown in the Fig. 7A-E.

Figure 8 presents the associations between the area of bony fusion and the duration of immobility, sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and the number of BrdU-positive cell. At 8 weeks post-surgery, the area of bony fusion was positively correlated with the duration of immobility. However, the area of bony fusion was negatively correlated with the sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and the number of BrdU-positive cell. The results of linear regression analysis revealed the area of bony fusion to be an independent variable affecting the duration of immobility, sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and the number of BrdU-positive cell. The linear regression equations for these relationships were shown in the Fig. 8A-E.

Discussion

TMJ ankylosis has significant effects on quality of life, and results in a poor physical and mental health in our previous data [1–3, 28]. To the best of the authors'knowledge, no previous study has been performed on the association between the depressive disorder and the TMJ ankylosis. The present study showed that depressive disorder was found in the rat model of traumatic TMJ bony ankylosis by micro-CT analysis, gross observation, behavioral tests, western blotting and immunocytochemistry tests. Moreover, the association between the severity of depressive disorder and the severity of TMJ bony ankylosis was verified and investigated.

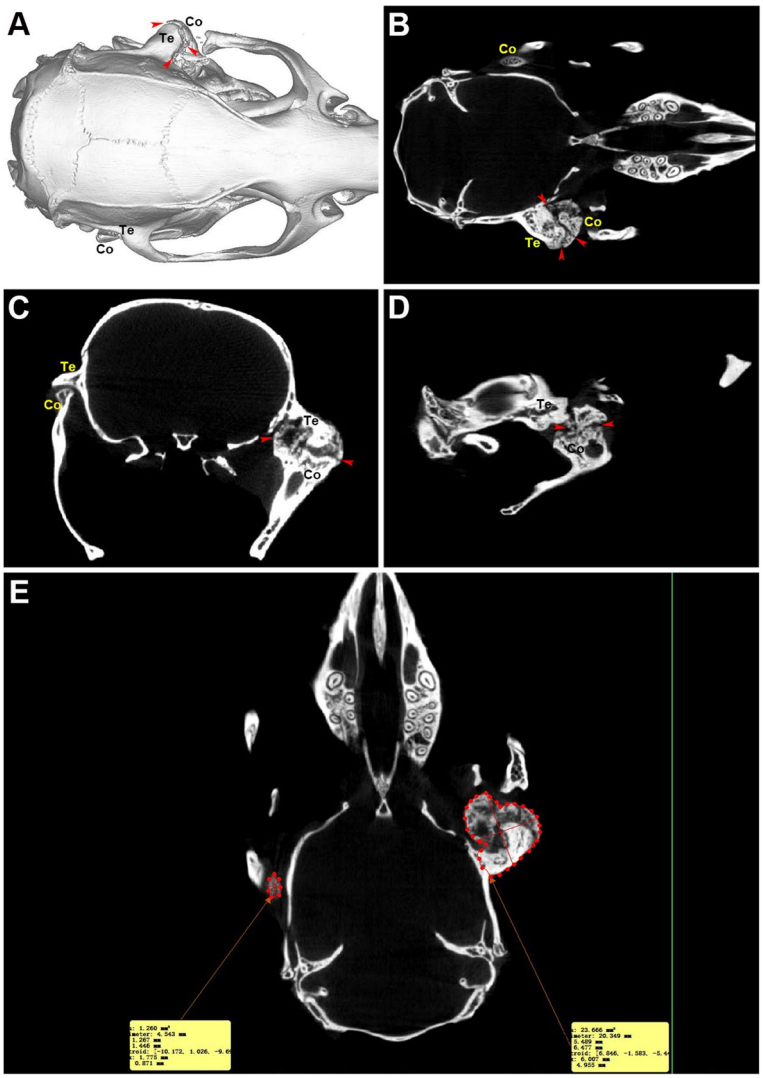

Fig. 3 TMJ complexes of experimental and sham side using micro-CT analysis at 8 weeks postoperatively. **A** 3D reconstruction of the micro-CT image shows newly formed bony fusion and narrowed joint space in the experimental side (red arrow), but not in the sham side; **B-D** axial, coronal, and sagittal micro-CT sections show disappeared joint space and more calcified bone callus formation through roughened upper and lower articular surfaces in the experimental side (red arrow) but not in the sham side; **E** The maximum areas of bony fusion and mass were measured in the axial micro-CT images on the experimental side and sham side, respectively

The animal model of TMJ bony ankylosis with SD rats established in the present study was found to be reliable. The key diagnostic indexes of TMJ bony ankylosis includes the gross observation, the presence of bony calcification images in the joint space, limitation of mouth opening, and so on [1–3]. In the test group, only the traumatic TMJ complexes with narrowed joint space developed TMJ bony ankylosis that a raised hard mass of the TMJ external appearance was observed and felt in the experimental side at 8 weeks post-surgery, but not in the sham side. Many calcificated bridges of bony fusion area were observed through the joint space in the 3D micro-CT in the present study, and the joint space of bony fusion area almost disappeared, the bony fusion areas of experimental side were obviously larger than the bony mass of sham side in the test group. Additionally, the body weight and PMMO of the test group were significantly lower than that of the control group at 8 weeks after surgery. These findings were in line with our previous study [2, 3].

The present study demonstrated that the SD rats of TMJ bony ankylosis showed representative depressive-like behaviors at 8 weeks after surgery. The forced swimming test and the sucrose preference test are used

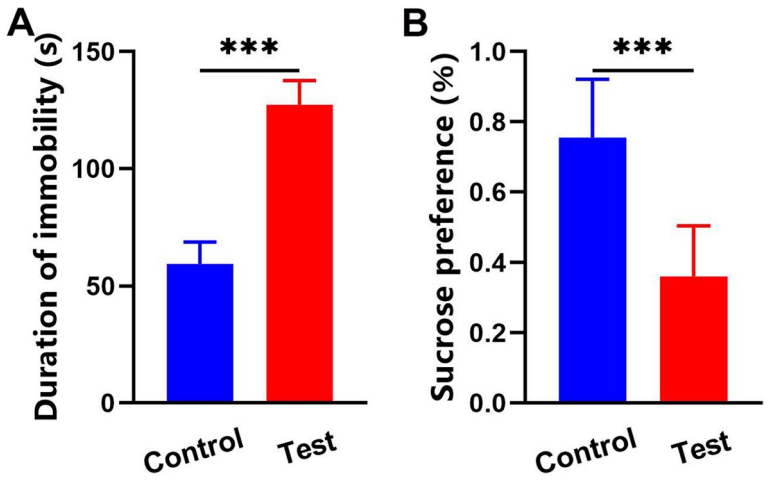

Fig. 4 Comparison of the behavioral changes with the SD rats between control group and test group at 8 weeks postoperatively. **A** Comparison of the duration of immobility in the forced swim test; **B** Comparison of the sucrose preference in sucrose preference test; Data represent mean ± SD; *,**,***: $p < 0.05, 0.01, 0.001$

as the measurement of depressive disorder [22, 29, 30]. The immobility of behavioral responses is total absence of movement or small movements of one of the mice posterior paws that do not produce displacement, the immobility time is used as a measurement of depressive-like behaviors in the forced swimming test [23, 31, 32], and higher level of immobility behavior in the forced swim test is associated to an increased "depression"-like behavior [23, 32, 33]. In the present study, the duration of immobility in the forced swimming test significantly increased in the test group than that in the control group. These results demonstrated that the rats were suffered from anhedonia and were diagnosed with depressive disorder, which is similar with previous findings that post-traumatic stress-induced depression is associated with increased duration of immobility [30, 34]. Furthermore, the sucrose preference test has been used as another measurement of the severity of anhedonia, which is inferred activation of the pleasure response to depression-like behaviour, and anhedonia is a core symptom of depressive disorder [24, 29, 35, 36]. In the present study, the sucrose preference significantly decreased in the test group than that in the control group, which is consistent with previous findings that stress-induced depressive disorder is associated with decreased sucrose preference [29, 30, 35]. These findings also verified that the anhedonic rats were suffered from depressive disorder. Therefore, these results suggest that the diagnosis of depressive disorder with SD rats in the animal model of TMJ bony ankylosis was found to be reliable using SPT and FST in the present study.

The present study found that the hippocampal expression of the CB1 receptor protein and the number of BrdU-positive cells significantly decreased in the dentate gyrus of test group compared to that of control group at 8 weeks after surgery. The CB1 receptors is an essential index for evaluating depressive disorder, the expression number of CB1 receptor protein is closely associated with depressive disorder, which reflects hippocampal neuroprotection and neurogenesis [30, 37, 38]. The CB1 receptors are widely expressed throughout the hippocampal formation, which are particularly dense in the dentate gyrus [25, 30, 35, 39]. Our previous study showed that the CB1 receptors and mean optical density of CB1 receptor protein were involved in the depressive disorder [26]. Previous paper reported that the CB1 receptors played an important role in a number of forms of long-term depressive disorder [39]. The chronic activation of CB1 receptors could increase neurogenesis and hippocampal progenitor proliferation, which was associated with the antidepressive action [40]. Our previous data found that reduced CB1 receptors was closely related with depressive-like behaviors [24]. The present study showed that the expression of CB1 receptor protein and mean optical density of CB1 receptor protein were significantly lower in the hippocampus of test group than that in the hippocampus of control group, the results showed that the SD rats of TMJ bony ankylosis were initially diagnosed with depressive disorder. The hippocampal expression of the BrdU-positive cells is another important index for evaluating depressive disorder. BrdU has become a marker for newly generated cells, and the expression of BrdU reflects the hippocampal neurogenesis and neuroprotection in local neural networks, which is closely associated with depressive disorder [35]. The BrdU-positive neurons in the dentate gyrus significantly

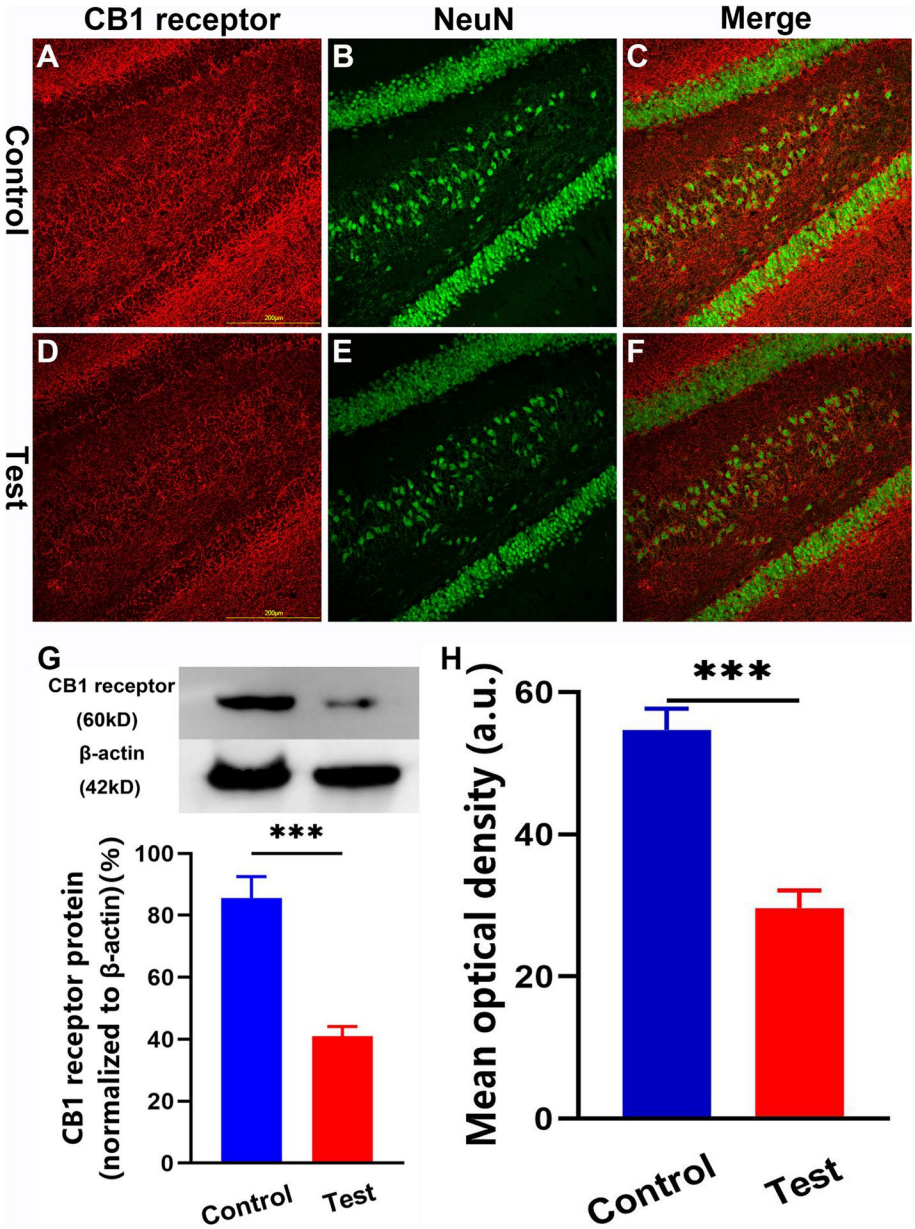

Fig. 5 Western Blotting (WB) detection and Immunocytochemistry analyse of CB1 receptor protein and mean optical density of CB1 receptor protein with the hippocampuses between control group and test group. **A-C** Representative microphotographs of CB1 receptor expression in the hippocampus of control group; **D-F** Representative microphotographs of CB1 receptor expression in the hippocampus of test group; **G** Densitometric analysis and representative bands comparison for the protein expression of CB1 receptor between control group and test group; **H** Comparison of the mean optical density of CB1 receptor protein in the hippocampus of control group and test group; Data represent mean ± SD; *,**,***: $p < 0.05, 0.01, 0.001$

decreased in the depressive rats [26, 31]. The anhedonia and behavioral despair were accompanied by a reduced expression of BrdU-positive cell [31, 35, 40]. The present study found that the hippocamal expression of the BrdU-positive cell was significantly lower in the dentate gyrus of test group than that in the dentate gyrus of control group, the results also showed that the SD rats of TMJ bony ankylosis were initially diagnosed with depressive disorder. Therefore, the diagnosis of depressive disorder with SD rats in the animal model of TMJ bony ankylosis was also verfied to be reliable using western blotting and immunocytochemistry in the present study.

The present study verified that there was a linear relationship between the PMMO and the severity of

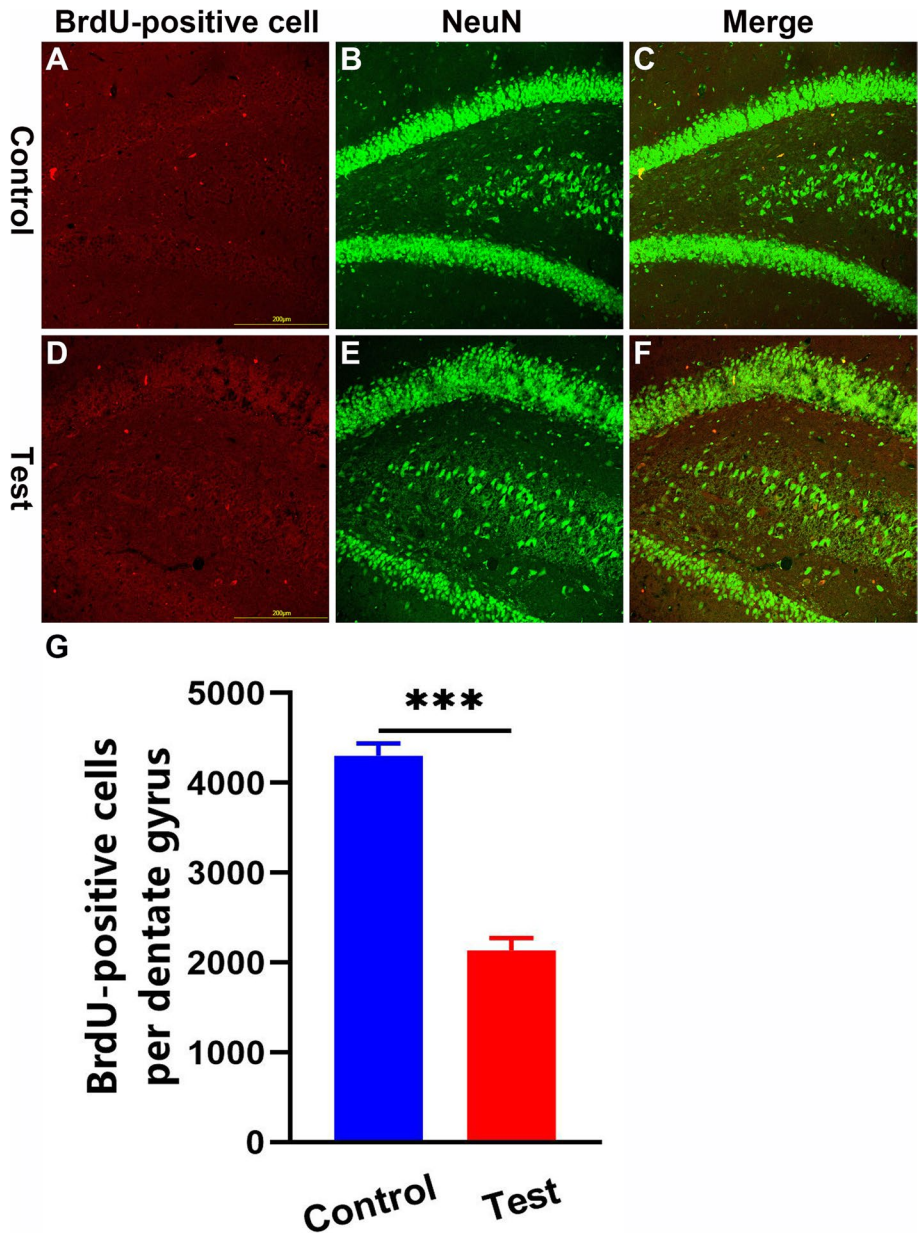

Fig. 6 Immunocytochemistry analyse of BrdU-positive cell expression with the hippocampus between control group and test group. **A-C** Representative microphotographs of BrdU-positive cells in the hippocampus of control group; **D-F** Representative microphotographs of BrdU-positive cells in the hippocampus of test group; **G** Comparison of the expression of BrdU-positive cells between the dentate gyrus of control group and that of test group; Data represent mean ± SD; *,**,***: $p < 0.05, 0.01, 0.001$

depressive disorder. The PMMO was another important index for evaluating TMJ bony ankylosis in our prvious reports [1, 3]. Our previous data demonstrated that the PMMO was important to evaluate the severity of TMJ bony ankylosis [2]. Similarly, the duration of immobility, sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and BrdU-positive cells per dentate gyrus were the important indexes for evaluating the severity of depressive disorder in our previous study [24–26]. In the present study, the results of the linear regression analysis revealed that duration of immobility, sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and BrdU-positive cells per dentate gyrus were variables affecting the PMMO at 8 weeks post-surgery. Specifically, the duration of immobility was negatively correlated with

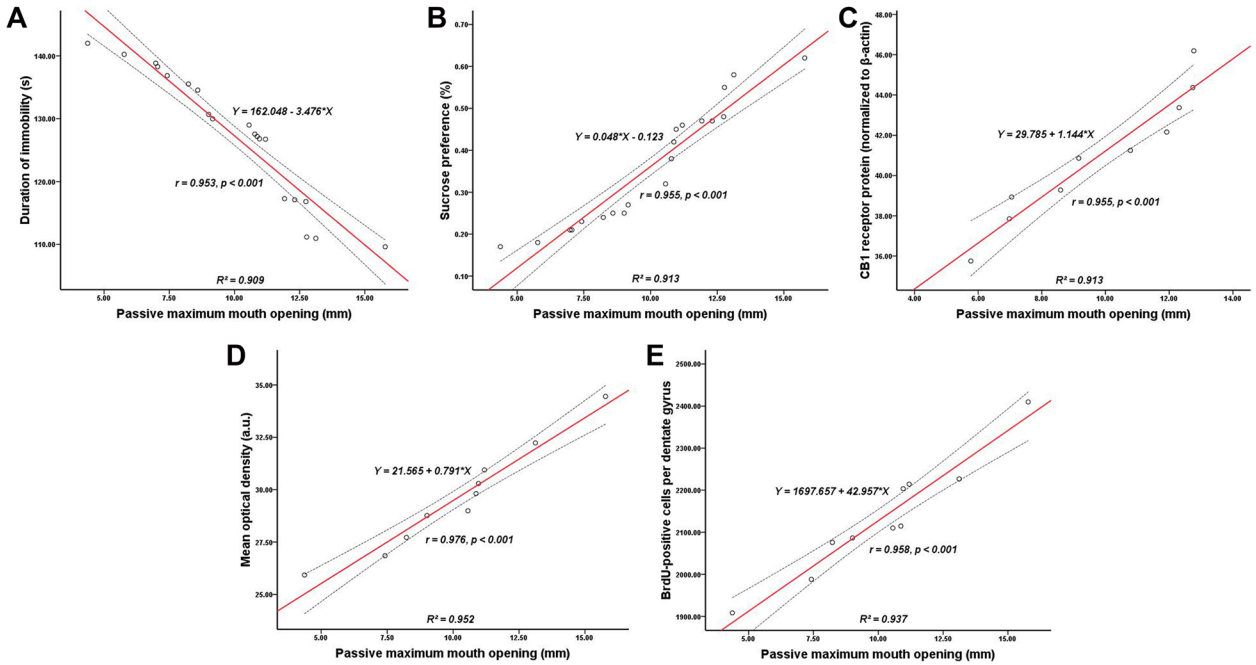

Fig. 7 The association between passive maximum mouth opening (PMMO) and the indicators of depressive disorder using linear regression analysis at 8 weeks postoperatively. **A** PMMO was both negatively correlated with the duration of immobility; **B-E** PMMO was positively correlated with sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, BrdU-positive cells

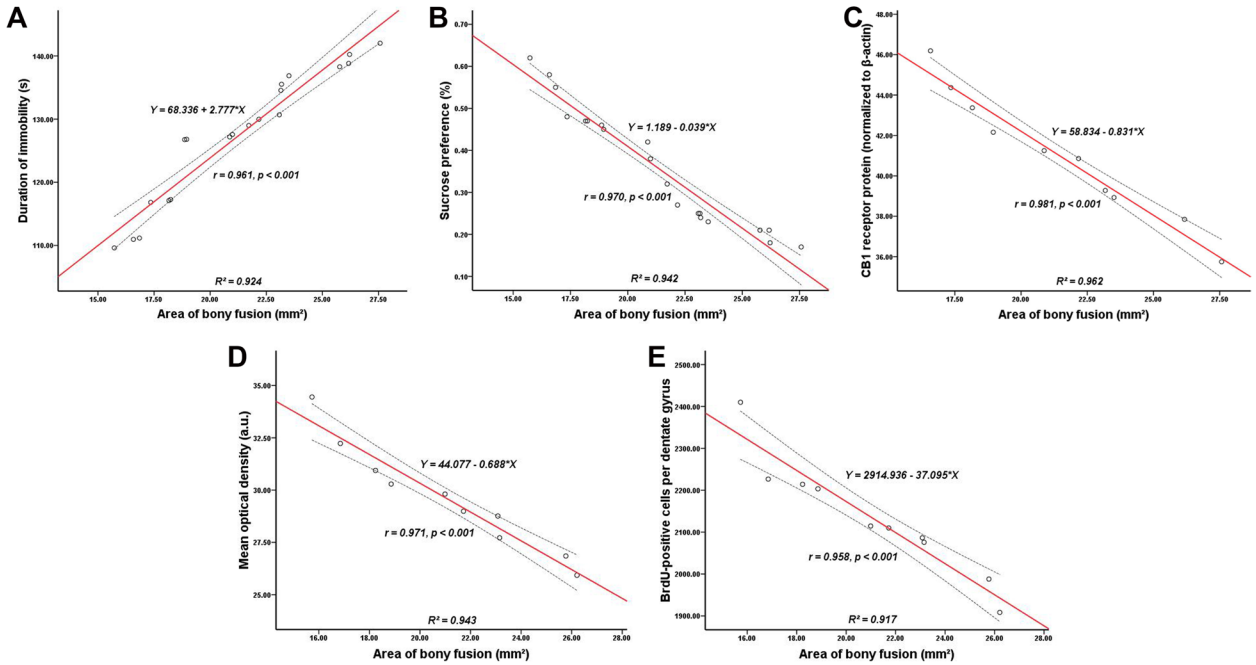

Fig. 8 The association between area of bony fusion and the indicators of depressive disorder using linear regression analysis at 8 weeks postoperatively. **A** Area of bony fusion was positively correlated with the duration of immobility; **B-E** Area of bony fusion was both negatively correlated with sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, BrdU-positive cells

PMMO, indicating that PMMO increased as the duration of immobility decreased. On the contrary, sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and BrdU-positive cells per dentate gyrus were positively correlated with PMMO, indicating that PMMO increased as sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and BrdU-positive cells per dentate gyrus increased. Therefore, the more severe limitation of PMMO, the more severity of TMJ bony ankylosis, the more severity of depressive disorder.

The present study also found that there was a linear relationship between the area of bony fusion on axial CT image and the severity of depressive disorder. Micro-CT, spiral-CT, and cone-beam CT are the important diagnostic tools for TMJ bony ankylosis, which are used to measure and analyze the area and volume of bony fusion area [1, 3, 12, 18]. Our previous data demonstrated that the area of bony fusion on axial CT image was an essential index to evaluate the severity of TMJ bony ankylosis [2]. Moreover, Our previous study found that the duration of immobility, sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and BrdU-positive cells per dentate gyrus were the important indexes for evaluating the severity of depressive disorder [24, 26]. In the present study, the results of the linear regression analysis revealed that duration of immobility, sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and BrdU-positive cells per dentate gyrus were variables that affected area of bony fusion at 8 weeks post-surgery. Specifically, the duration of immobility was positively correlated with area of bony fusion, indicating that area of bony fusion increased as the duration of immobility increased. On the contrary, sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and BrdU-positive cells per dentate gyrus were negatively correlated with area of bony fusion, indicating that area of bony fusion increased as sucrose preference, CB1 receptor protein, mean optical density of CB1 receptor protein, and BrdU-positive cells per dentate gyrus decreased. Therefore, the greater the area of bony fusion, the more severity of TMJ bony ankylosis, the more severity of depressive disorder. Thus, we deduced that the synergy between the more area of bony fusion, the more limited PMMO and the more severity of depressive disorder may seriously impact on quality of life, and poor quality of life may further worsen TMJ bony ankylosis and depressive disorder.

Nevertheless, the underlying mechanism underpinning this is complicated and unclear. Next, we will plan to observe the course of animal depressive disorder continuously over time, and further explore the detailed mechanism of CB1 receptor protein and BrdU-positive newly generated neurons in the animal model of TMJ bony ankylosis, and then prevent the occurrence of TMJ bony ankylosis by regulating the expression of CB1 receptor protein and BrdU-positive cells. Previous study reported that the electroacupuncture treatment ameliorates post-traumatic stress depressive-like behavior [18, 30, 34]. Furthermore, antidepressants or electroacupuncture will be considered to treat the animals of traumatic TMJ bony ankylosis whether or not can relieve or eliminate the symptoms of traumatic TMJ bony ankylosis. The therapeutic effects of antidepressants or electroacupuncture with post-traumatic depressive disorder and traumatic TMJ bony ankylosis will be carefully assessed using animal model. The therapeutic effects and specific mechanisms of antidepressants or electroacupuncture are expected to open up new ideas for the treatment of human TMJ ankylosis in the future.

Some limitations should be mentioned in the present study. Firstly, the SD rats of traumatic TMJ bony ankylosis were only assessed at 8 weeks after surgery, the course of depressive disorder was not observed continuously over time. Secondly, the diagnosis of depressive disorder in the present study was not further validated by anti-depressive drugs. Thirdly, the animals in the control group did not undergo sham surgery. The postoperative effects of ankylosis inducing surgeries and sham surgery on the contralateral TMJ may be confounding factors. Fourthly, the animals in the test group received pentazocine analgesic after surgery, the pentazocine is a weak competitive opioid antagonist at the μ receptor and an agonist at the κ 1 and κ 2 receptors. Its use may represent another confounding factor in the study, since the drug acts on the central nervous system and the animals in the control group did not receive medication. Fifthly, the research conclusion of the present data is extended to human being duo to limitations of animal model and certain racial differences. Therefore, the present results cannot be completely transferred to the human situation to serve purposes of diagnosis and treatment. A further study will be performed to overcome these shortcomings in the future.

Conclusions

The present study demonstrated that depressive disorder was found in the rat model of traumatic TMJ bony ankylosis. The severity of TMJ bony ankylosis correlated with the severity of depressive disorder.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12903-025-05886-7.

Supplementary Material 1.

Acknowledgements
Thank you to all the participants who participated in the study.

Authors' contributions
Tiange Deng, Yang Xue, and Yiming Wang contributed equally in the design of this study. Tiange Deng and Lei Wang performed the present study. Tiange Deng and Yang Xue wrote the manuscript. Xiang Guo, Zhen Ma, and Hongzhi Zhou collected the data. Zaiyong Yang, Zhengwu Peng, Xiangxiang Hu, and Ning Li analyzed the data. Xiangxiang Hu and Ning Li corrected the English version of this manuscript. Kaijin Hu and Lei Wang reviewed and edited the manuscript. All authors have read and approved the submission of the manuscript.

Funding
This study was supported by the National Natural Science Foundation of China (Grant No. 81970954), the New Technology and New Project of the Third Affiliated Hospital of Air Force Military Medical University (Grant No. LX2021 - 332), the General Project of Social Development Field of Shaanxi Natural Science Foundation (Grant No. 2024SF-YBXM- 263), and the General Project of Key Research and Development Field of Shaanxi Natural Science Foundation (Grant No. 2024SF-YBXM-434).

Data availability
The datas are available upon reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate
All experiments described in this study were approved by the animal care and ethics committee of the school of stomatology (ID 2020–0950). Animal maintenance and all experimental procedures were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023) and the ARRIVE guidelines on the Care and Use of Experimental Animals.

Consent for publication
No applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]State Key Laboratory of Oral & Maxillofacial Reconstruction and Regeneration, National Clinical Research Center for Oral Diseases, Shaanxi Clinical Research Center for Oral Diseases, Department of Oral and Maxillofacial Surgery, School of Stomatology, The Fourth Military Medical University, Xi'an 710032, China. [2]Oral Medicine Teaching and Research Office of the War Rescue Medical Department of the, Noncommissioned Officer Schoolaq, Army Medical University, Shijiazhuang 050081, China. [3]Department of Psychiatry, Xijing Hospital, the Fourth Military Medical University, Xi'an 710032, China. [4]Division of Oral and Craniofacial Health Sciences, University of North Carolina Adams School of Dentistry, Chapel Hill, NC 27514, USA. [5]School of Stomatology, Jinzhou Medical University, Jinzhou 121001, China. [6]Department of Stomatology, School of Stomatology, Xi'an Medical University, the Third Affiliated Hospital of Xi'an Medical University, 1 Xinwang Road, Xi'an 710021, China. [7]Department of Female Mental Health, Xi'an Mental Health Center, Xi'an Institute of Mental Health, Shaanxi Mental Health Center, Dong Hangtian Road, Xi'an 710061, China.

Received: 4 July 2024 Accepted: 26 March 2025
Published online: 11 April 2025

References

1. Deng TG, Liu CK, Liu P, Zhang LL, Wu LG, Zhou HZ, et al. Influence of the lateral pterygoid muscle on traumatic temporomandibular joint bony ankylosis. BMC Oral Health. 2016;16(1):e62.
2. Deng TG, Liu CK, Wu LG, Liu P, Wang JJ, Sun XZ, et al. Association between maximum mouth opening and area of bony fusion in simulated temporomandibular joint bony ankylosis. Int J Oral Maxillofac Surg. 2020;49(3):369–76.
3. Ma Z, Wang Y, Xue Y, Zhang W, Li D, Li Y, et al. Traumatic temporomandibular joint bony ankylosis in growing rats. BMC Oral Health. 2022;22(1):e585.
4. Shirani G, Arshad M, Mahmoudi X. A new method of treatment of temporomandibular joint ankylosis with osteodistraction using the sh-device: a case report. J Dent (Tehran). 2018;15(1):63–8.
5. Park LT, Zarate CA. Depression in the primary care setting. N Engl J Med. 2019;380(6):559–68.
6. Song J, Kim YK. Animal models for the study of depressive disorder. CNS Neurosci Ther. 2021;27(6):633–42.
7. Williams ES, Mazei-Robison M, Robison AJ. Sex differences in major depressive disorder (MDD) and preclinical animal models for the study of depression. Cold Spring Harb Perspect Biol. 2022;14(3):1–18.
8. Okereke OI, Vyas CM, Mischoulon D, Chang G, Cook NR, Weinberg A, et al. Effect of long-term supplementation with marine omega-3 fatty acids vs placebo on risk of depression or clinically relevant depressive symptoms and on change in mood scores: a randomized clinical trial. JAMA. 2021;326(23):2385–94.
9. Bocquet E, Mercier M, Moreau A, Ferri J, Danguy M. A particular temporomandibular disorder: the temporomandibular joint ankylosis. basic and clinical concepts. Orthod Fr. 2014;85(4):363–75.
10. Woo J, Lee CR, Choi JY. Temporomandibular joint ankylosis in williams syndrome patient: an insight on the function of elastin in temporomandibular joint disorder. J Korean Assoc Oral Maxillofac Surg. 2022;48(3):178–81.
11. Beret M, Nicot R, Gutman L, Ferri J. Quality of life after total temporomandibular joint prothesis surgery. J Craniofac Surg. 2022;33(7):2134–7.
12. Zhao L, Xiao E, He L, Duan D, He Y, Chen S, et al. Reducing macrophage numbers alleviates temporomandibular joint ankylosis. Cell Tissue Res. 2020;379(3):521–36.
13. Liu Y, Hu P, Zheng Z, Zhong D, Xie W, Tang Z, et al. Photoresponsive vaccine-like CAR-M system with high-efficiency central immune regulation for inflammation-related depression. Adv Mater. 2022;34(11): e2108525.
14. Zúñiga-Herrera ID, Herrera-Atoche JR, Aguilar-Pérez FJ, Escoffié-Ramírez M. Malocclusion complexity in patients with myofascial pain with or without mouth-opening limitation: a case-control study. Biomed Res Int. 2022;2022: e3594246.
15. Liao HY, Satyanarayanan SK, Lin YW, Su KP. Clinical efficacy and immune effects of acupuncture in patients with comorbid chronic pain and major depression disorder: a double-blinded, randomized controlled crossover study. Brain Behav Immun. 2023;110:339–47.
16. Cox N, Hawarden A, Bajpai R, Farooq S, Twohig H, Muller S, et al. The relationship between pain and depression and anxiety in patients with inflammatory arthritis: a systematic review protocol. Rheumatol Int. 2024;44(3):435–40.
17. Cui M, Ji R, Song L, Wang X, Pan X, Han Y, et al. Neuronal and molecular mechanisms underlying chronic pain and depression comorbidity in the paraventricular thalamus. J Neurosci. 2024;44(13): e1752232024.
18. Songphaeng T, Lapmanee S, Bhubhanil S, Momdee K, Rojviriya C, Kitsahawong K, et al. Atomoxetine and escitalopram migrate the derangement of the temporomandibular joint morphologic and histologic changes in rats exposed to stress-induced depression. J Oral Sci. 2023;65(4):219–25.
19. Florjański W, Orzeszek S. Role of mental state in temporomandibular disorders: a review of the literature. Dent Med Probl. 2021;58(1):127–33.
20. Xu L, Cai B, Fan S, Lu S, Dai K. Association of oral behaviors with anxiety, depression, and jaw function in patients with temporomandibular disorders in China: a cross-sectional study. Med Sci Monit. 2021;27: e929985.
21. Sharma VK, Rattan V, Rai S, Sharma P. Development and validation of temporomandibular joint ankylosis quality of life questionnaire. J Craniomaxillofac Surg. 2020;48(8):779–85.
22. Slattery DA, Cryan JF. Using the rat forced swim test to assess antidepressant-like activity in rodents. Nat Protoc. 2012;7(6):1009–14.
23. Wang T, Niu K, Fan A, Bi N, Tao H, Chen X-T, et al. Dietary intake of polyunsaturated fatty acids alleviates cognition deficits and depression-like behaviour via cannabinoid system in sleep deprivation rats. Behav Brain Res. 2020;384: e112545.

24. Xue SS, Xue F, Ma QR, Wang SQ, Wang Y, Tan QR, et al. Repetitive high-frequency transcranial magnetic stimulation reverses depressive-like behaviors and protein expression at hippocampal synapses in chronic unpredictable stress-treated rats by enhancing endocannabinoid signaling. Pharmacol Biochem Behav. 2019;184: e172738.
25. Fang G, Wang Y. Effects of rTMS on hippocampal endocannabinoids and depressive-like behaviors in adolescent rats. Neurochem Res. 2018;43(9):1756–65.
26. Wang HN, Wang L, Zhang RG, Chen YC, Liu L, Gao F, et al. Anti-depressive mechanism of repetitive transcranial magnetic stimulation in rat: the role of the endocannabinoid system. J Psychiatr Res. 2014;51:79–87.
27. Kikuchi A, Shimizu K, Nibuya M, Hiramoto T, Kanda Y, Tanaka T, et al. Relationship between post-traumatic stress disorder-like behavior and reduction of hippocampal 5-bromo-2'-deoxyuridine-positive cells after inescapable shock in rats. Psychiatry Clin Neurosci. 2008;62(6):713–20.
28. Liu CK, Meng FW, Tan XY, Xu J, Liu HW, Liu SX, et al. Clinical and radiological outcomes after treatment of sagittal fracture of mandibular condyle (SFMC) by using occlusal splint in children. Br J Oral Maxillofac Surg. 2014;52(2):144–8.
29. Shen CJ, Zheng D, Li KX, Yang JM, Pan HQ, Yu XD, et al. Cannabinoid CB1 receptors in the amygdalar cholecystokinin glutamatergic afferents to nucleus accumbens modulate depressive-like behavior. Nat Med. 2019;25(2):337–49.
30. Peng ZW, Zhou CH, Xue SS, Yu H, Shi QQ, Xue F, et al. High-frequency repetitive transcranial magnetic stimulation regulates neural oscillations of the hippocampus and prefrontal cortex in mice by modulating endocannabinoid signalling. J Affect Disord. 2023;331:217–28.
31. Zhang L, Tang W, Ouyang Y, Zhang M, Li R, Sun L, et al. N-palmitoylethanolamine modulates hippocampal neuroplasticity in rats with stress-induced depressive behavior phenotype. Eur J Pharmacol. 2023;957: e176041.
32. Rezaie M, Nasehi M, Vaseghi S, Alimohammadzadeh K, Vaghar MI, Mohammadi-Mahdiabadi-Hasani M-H, et al. The interaction effect of sleep deprivation and cannabinoid type 1 receptor in the CA1 hippocampal region on passive avoidance memory, depressive-like behavior and locomotor activity in rats. Behav Brain Res. 2021;396: e112901.
33. Branchi I, D'Andrea I, Sietzema J, Fiore M, Fausto VD, Aloe L, et al. Early social enrichment augments adult hippocampal BDNF levels and survival of BrdU-positive cells while increasing anxiety- and "depression"-like behavior. J Neurosci Res. 2006;83(6):965–73.
34. Xue SS, Zhou CH, Xue F, Liu L, Cai YH, Luo JF, et al. The impact of repetitive transcranial magnetic stimulation and fluoxetine on the brain lipidome in a rat model of chronic unpredictable stress. Prog Neuropsychopharmacol Biol Psychiatry. 2020;102: e109946.
35. Mao L, Lv FF, Yang WF, Zhang TF, Li ZC, Li DQ, et al. Effects of baihui electroacupuncture in a rat model of depression. Ann Transl Med. 2020;8(24): e1646.
36. Yu J, Ren L, Min S, Lv F, Luo J, Li P, et al. Inhibition of CB1 receptor alleviates electroconvulsive shock-induced memory impairment by regulating hippocampal synaptic plasticity in depressive rats. Psychiatry Res. 2022;317: e114751.
37. Egaña-Huguet J, Saumell-Esnaola M, Achicallende S, Soria-Gomez E, Río IB-D, Caño GGD, et al. Lack of the transient receptor potential vanilloid 1 shifts cannabinoid-dependent excitatory synaptic plasticity in the dentate gyrus of the mouse brain hippocampus. Front Neuroanat. 2021;15:e701573.
38. Martín-Sánchez A, García-Baos A, Castro-Zavala A, Alegre-Zurano L, Valverde O. Early-life stress exacerbates the effects of WIN55,212–2 and modulates the cannabinoid receptor type 1 expression. Neuropharmacology. 2021;184:e108416.
39. Fontaine CJ, Gräfe EL, Pinar C, Río IB-D, Grandes P, Christie BR. Endocannabinoid receptors contribute significantly to multiple forms of long-term depression in the rat dentate gyrus. Learn Mem. 2020;27(9):380–9.
40. Campos AC, Ortega Z, Palazuelos J, Fogaça MV, Aguiar DC, Díaz-Alonso J, et al. The anxiolytic effect of cannabidiol on chronically stressed mice depends on hippocampal neurogenesis: involvement of the endocannabinoid system. Int J Neuropsychopharmacol. 2013;16(6):1407–19.

Publisher's Note