Increased expression of TRPV1 in the central nucleus of the amygdala is involved in orthodontic pain during experimental tooth movement in rats

Rui Wang
College of Stomatology, Xi`an Jiaotong University
Dan Fang
College of Stomatology, Xi`an Jiaotong University
Shuyun Lu
College of Stomatology, Xi`an Jiaotong University
Yunfei Jia
College of Stomatology, Xi`an Jiaotong University
Yining Guo
College of Stomatology, Xi`an Jiaotong University
Xianya Liu
College of Stomatology, Xi`an Jiaotong University
Hu Qiao (qiaohu_xjtu@163.com)
College of Stomatology, Xi`an Jiaotong University https://orcid.org/0000-0003-2323-4881

Research Article

Keywords: Orthodontic pain, central nucleus of the amygdala, TRPV1, behaviour assessment, central regulation

Posted Date: January 9th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2421619/v1

Abstract

Pain is one of the most common adverse reactions during orthodontic treatment, which troubles patients a lot. Transient receptor potential vanilloid 1 (TRPV1) plays a crucial role in pain transmission and is expressed in the peripheral nervous system, but there is a paucity of literature on the roles of TRPV1 in the central nervous system. The central amygdala (CeA) integrates multiple sensory signals including nociceptive sensory information. However, how the involvement of TRPV1 in the CeA in orthodontic pain has not been investigated. To explore this, we constructed an experimental tooth movement (ETM) model using precision springs and evaluated pain behaviour based on face-grooming and the rat grimace scale (RGS). TRPV1 expression in the CeA was evaluated using immunofluorescence and western blotting. Face-grooming and RGS score peaked on day 1 then decreased gradually to baseline levels on day 7. Immunofluorescence and western blotting analysis revealed that TRPV1 expression in the CeA increased after ETM. Furthermore, changes in TRPV1 expression in the CeA were positively associated with RGS behaviour. Our findings suggest that TRPV1 in the CeA is modulated by ETM and is involved in tooth-movement pain, providing a new understanding of central regulation on orthodontic pain.

Introduction

Pain is defined as an unpleasant feeling and emotional experience accompanied by actual or potential tissue damage [1]. Most orthodontic patients experience pain during the treatment process[2], which can lead to major dissatisfaction and discontinuation of long-term treatment[3, 4]. Most research on orthodontic pain have focused on peripheral mechanisms, and central mechanisms remain poorly defined. Orthodontic pain is thought to be caused by the stimulation of periodontal and pulp tissues, which can be activated by orthodontic force and local inflammatory mediators. These impulses are transmitted to the trigeminal ganglion through Aδ and C fibres, and then projected to the central nervous system[5, 6].

Pain is regulated by several cerebral structures, which are involved in detecting, expressing, and regulating pain. In particular, the amygdala plays a key role in pain perception among structures involved in pain sensation[7]. In both humans and rodents, the amygdala is activated in response to pain[8, 9]. Among amygdaloid nuclei, the central nucleus of the amygdala (CeA) integrates multiple sensory signals including nociceptive sensory information. The CeA processes nociceptive input from the spinal-parabrachial-amygdala pathway and transmits the resulting information to the hypothalamus, substantia innominate dorsalis and brainstem nuclei that control responses to defensive behaviour[10, 11]. Our previous research revealed the role of the CeA in orthodontic pain during tooth movement[12]. Specifically, after electrolytic destruction of the CeA, pain during tooth movement was significantly reduced. Moreover, various pain-related mediators and receptors play key roles in the process of orthodontic pain and pain information transmission. Transient receptor potential vanilloid type 1 (TRPV1) is a polymodal noci-transducer that is expressed widely in the cortex, hippocampus, CeA, dentate gyrus, hypothalamus, striatum, thalamus, nucleus of the trigeminal nerve and inferior olive[13–16]. TRPV1 is activated by exogenous chemical and physical stimuli and is considered a novel target for the control of

inflammatory pain[17]. TRPV1 in the peripheral nervous system has been reported, but few focused on the roles of TRPV1 in the central nervous system, thus our study may show the role of TRPV1 in the CeA on regulating orthodontic pain.

Based on our previous findings[12], this study aimed to investigate pain behaviour and TRPV1 expression in the CeA during tooth movement using behavioural and molecular biology techniques in order to elucidate the complex pain regulation network and identify effective interventional measures for orthodontic pain.

Materials And Methods

Experimental design and animals

8W male Sprague-Dawley rats weighing between 200 and 250 g were obtained from the Experimental Animal Centre of Xi'an Jiaotong University Health Science Centre (Xi'an, China). Rats were nursed in standard cages with food and water on a 12-h light/12-h dark cycle in a temperature-controlled (21 ± 1.5°C) room. Rats were allowed to acclimatise to the housing conditions for at least 5 d before commencement of experiments. A total of 72 rats were divided into four groups based on force depending on the extension distance of precision spring as follows: control, 30 g, 50 g, and 80 g groups. Each group was examined at 0 h, 4 h, 1 d, 3 d, 5 d, and 7 d after the force. Face-grooming behaviour and the rat grimace scale (RGS) were used to observe behavioural responses to pain. Tooth movement distance was evaluated using micro-CT. TRPV1 expression in the CeA was investigated using immunofluorescence and western blotting. Every effort was made to minimise animal discomfort, in accordance with the ethical guidelines for animal research[18]. Experimental procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care Committee of Xi`an Jiaotong University.

Experimental Tooth Movement (ETM)

Rats were anesthetised with sodium pentobarbital (i.p.; 60 mg/kg). A fixed nickel titanium alloy closed-coil spring appliance was constructed to perform mesial movement of the right maxillary first molar[19]. Specifically, the closed-coil spring hooked the right maxillary first molar and incisors. A shallow groove was made on the neck of the upper incisors, and a glass ionomer was used as a cover to enhance retention.

Face-grooming Behaviour

Rats were monitored for directed face-grooming behaviour in transparent plastic cages (40 cm × 40 cm × 40 cm) placed in a room with 45 dB background noise between 9 AM and 12 AM. Rats were allowed to acclimatise to the housing conditions for at least 15 min before recordings were performed. Behaviour

was recorded by two cameras for a period of 10 min at each time point, and each rat was recorded three times at 20 min intervals. The test session was subsequently analysed by two independent observers who were blinded to the treatment. The average of three estimations was used to yield a mean value for each animal.

RGS

Cameras were placed on both sides of a transparent box, and each rat was continuously recorded for 30 min. A clear image of the rat's face was captured every 3 min and scored. In total, 10 photos were captured and encoded for each rat. RGS scoring was performed by two experimenters in a blinded manner[20].

Micro-CT

Tooth movement distance was measured using micro-CT. The rat’s head was placed into a position that was symmetrically aligned to the three spatial axes within a recordable cylindrical volume of 4 cm × 4 cm × 4 cm. The distance of the most prominent point from the distal side of the first molar to mesial side of the second molar of the maxillary was measured and analysed.

Immunohistochemistry

The distribution of TRPV1 receptors in the CeA was analysed using immunofluorescence and western blot after ETM in control and 50 g-force experimental rats (n = 3 rats per group). Rats were killed with an overdose of 10% chloral hydrate and perfused with 150 mL of saline solution followed by 500 mL of 4% paraformaldehyde. Brains were removed and postfixed for 12–24 hours in 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The brains were then placed in a 30% sucrose solution for another 72 h. Following sucrose cryoprotection, 25-μm sections were cut on a freezing sliding microtome. Sections were stored at 4°C until the tissue was prepared for immunofluorescence[21].

After three washes in phosphate-buffered saline (PBS), tissue sections were incubated for 15 min at room temperature (23 ± 3°C) in a blocking solution of Quickblock™ Blocking Buffer for Immunol Staining (Beyotime Biotechnology, China). Slides were incubated with rabbit anti-TRPV1 IgG (1:500 dilution; Alomone, Israel) at 4 ℃ for 48 h. The specificity of the goat anti rabbit IgG antibody has been reported previously (Lin and others, 2020). The samples were then incubated with a 488-conjugated goat anti rabbit IgG (H + L) secondary antibody (1:500 dilution; Immunoway, American) for 4–6 h at room temperature. Sections were examined under a fluorescence microscope (Olympus, Tokyo, Japan) equipped with a digital camera.

Western Blot Analysis

Protein extraction of CeA tissue was conducted with RIPA lysis buffer and a protease inhibitor cocktail (Roche, Germany). BCA (bicinchoninic acid) method (Beyotime Biotechnology, China) was used to determine the protein concentration. A total of 30 μg of protein of each sample was separated in 10% TGX Stain-Free polyacrylamide gels (Bio-Rad, USA). Total protein data were obtained using a GelDoc Go Imaging System Ordering (Bio-Rad, USA) and transferred onto a membrane. After blocking with 5% skim milk powder at 25°C for 1 h, the following primary and secondary antibodies were used: polyclonal rabbit anti-TRPV1 (Santa Cruz, USA), goat-anti-rabbit IgG (1:5000 dilution; CST, American). The densities of the protein blots were analysed using Imagelab Software (Bio rad, USA). Total protein was used as an internal control to normalise the target protein levels.

Statistical analysis

Continuous data are expressed as mean ± standard deviation (SD). For multiple group comparisons, initial analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc Fisher's least significant difference (LSD) test. Comparisons between two groups were performed using the Student's t-test for paired data. Pearson correlation was used to determine the correlation between the intensity of TRPV1 expression (western blot) and behavioural performance. Differences were considered statistically significant at $P < 0.05$.

Results

Micro-CT and rate of tooth movement

Tooth movement is presented in Fig. 1. Tooth movement can be observed based on different magnitudes of force, and the movement distance increases with an increase in force. Before 5 days postoperatively, the force of 50 g was the fastest. We speculated that the force of 30 g was small, resulting in limited effects. In contrast, the force of 80 g was too large to move the tooth properly due to latent bone resorption. Micro-CT results revealed that applying a force of 80 g to the teeth in rats produced evident root resorption. Accordingly, 50 g was selected as the force magnitude for subsequent experiments.

Face-grooming behaviour After ETM With 50 G Force

In this study, three experimental groups of rats were established: blank control, sham (0 g), and 50 g groups (n = 8 per group). Changes in face-grooming activity of rats in each group were observed at 4 h, 1 d, 3 d, 5 d, and 7 d (Fig. 2). The analysis revealed that 50 g induced obvious pain behaviour, and the pain peaked at day 1.

RGD After ETM With 50 G Force

Changes in RGS score following initiation of ETM are presented in Fig. 3. The results exhibited a similar tendency to that of face-grooming behaviour.

Expression Of Trpv1 In The CeA After ETM

To investigate the expression of TRPV1 in the CeA after ETM, immunofluorescence analysis was performed. As shown in Fig. 4, TRPV1 immunofluorescence (indicated in green) was observed in the CeA. Compared to total protein, TRPV1 expression in the CeA was significantly differentially expressed. TRPV1 was most strongly expressed at 4 h and returned to baseline at 7 d (Fig. 5). A positive correlation was observed between RGS score and TRPV1 expression in the CeA. Results for the sham-treated (R^2 = 0.04749, P 0.05) and experimental (R^2 = 0.3628, P < 0.0175) groups are presented in Fig. 6.

Discussion

Pain is a common adverse reaction in the process of orthodontic treatment. Approximately 95% of patients will experience discomfort and pain during orthodontic treatment, which may result in anxiety and negatively impact their mental state. Indeed, some patients may even discontinue follow-up orthodontic treatment[22–24]. Adults generally spend approximately 2 years on orthodontic treatment, and there is a high demand for patient compliance during treatment. As pain is a key factor affecting patients' comfort, it is critical for patients to maintain good compliance during the course of treatment. Accordingly, it is essential to reduce pain associated with tooth movement to improve patient comfort and compliance while ensuring treatment quality.

Oral and maxillofacial pain is often evaluated by face-grooming activity, RGS coding and other evaluation methods[20, 25]. Our group used face-grooming activities and RGS coding to conduct a joint evaluation. Our analysis revealed that the two evaluation methods had reliability and repeatability. Face-grooming activities include different actions, such as mouth wiping, forelimb flailing, ear grasping, face-washing strokes, headshakes, paw licks and chin rubs. Mouth wiping is a directed face-grooming activity that can be reliably and reproducibly measured and is the most robust behavioural response for pain arising after ETM[25]. RGS coding predominantly analyses changes in facial expressions, including orbital tightening (eye closure or squeezing), nose / cheek flattening (lack of bulge on the top of the nose, flattened and elongated nose and beard pad and / or crease between the missing pad and cheek), ear changes (pointed ear that folds, curls and tilts forward or outward), and whisker changes (whiskers move forward and tend to bunch). This approach is also a reliable method for evaluating pain responses during orthodontic tooth movement[20, 26].After the establishment of the ETM model in SD rats, scores of face-grooming activities and RGS coding increased from 4 h to 1 d, peaked at 1 d, decreased from 3 d to 7 d, and returned to baseline levels at 7 d. These findings are consistent with previous research and clinical observations of orthodontic pain[27, 28], also implied pain occurrence derived from ETM.

In this study, the rate of tooth movement and pain were measured. Orthodontic tooth movement models using SD rats are generally established by placing a stainless-steel tension spring between the incisor

and molars, which relies on the force of the tension spring to produce tooth movement. Under the continuous action of a 50 g spring force, the first molars of rats could produce the effect of tooth movement on day 3, and the distance of tooth movement increased continuously with the passage of time. From day 1 to day 3, tooth movement was faster in the 30 g and 50 g groups than in the 80 g group, which could be due to excessive stress, osteocyte degeneration and necrosis in the 80 g group. After 7 days, tooth movement rate was significantly increased in the 30 g and 50 g groups, which may be due to the self-repair of periodontal tissue and active osteoclast phenomenon. In contrast, the basic stagnation of tooth movement in the 80 g group may be due to severe periodontal damage and necrotic tissue removal[29].

As the emotional integration centre of the body, the amygdala is closely associated with the production of pain emotion and its somatic responses (avoidance behaviour). The amygdala is considered a key nucleus connecting pain and emotion. As a critical structure of the limbic forebrain, the amygdala is a heterogeneous structure composed of approximately a dozen nuclei[30]. Amygdala activity is significantly increased in individuals exposed to anxiety-inducing environments and in those with anxiety disorders. The anatomical connections of the amygdala to other subcortical regions involved in emotional responses support its role in the processing of anxiety and fear associated with painful stimuli or experiences. The amygdala comprises the basolateral amygdala, CeA, medial amygdala, cortical amygdala, and other scattered nuclei. The CeA is the primary output nucleus from the amygdala and constitutes the main efferent amygdaloid nucleus that projects to the brainstem and hypothalamus. The CeA receives fibre projections from outside the CeA and has reciprocal fibre connections with these regions. The CeA integrates inputs from other amygdaloid nuclei, including nociceptive sensory information[11]. The CeA acts as an emotional integration centre and participates in the regulation of pain and anxiety. TRPV1 plays a role in the formation of pain sensitisation and neuropathic pain by interacting with inflammatory mediators and affecting the excitability of nerve fibres and neurons[31]. In this study, TRPV1 expression in the CeA of rats with orthodontic tooth movement was analysed. The results highlighted regularity in TRPV1 expression in the CeA among groups. Moreover, TRPV1 expression was positively correlated with RGS coding. These findings suggest that TRPV1 in the CeA may be involved in the exacerbation of orthodontic pain.

After establishing the ETM model, our analysis revealed that pain behaviour and TRPV1 expression in the CeA peaked at day 1. Indeed, there was a correlation between TRPV1 expression and pain behaviour induced by ETM. However, it remains unclear whether TRPV1 in the CeA is directly expressed in the central nervous system or transmitted from the peripheral nervous system to the central nervous system, which warrants further research.

In conclusion, this study established an ETM model by applying 50 g force and analysed the expression of TRPV1 in the CeA. Our results demonstrated that TRPV1 expression in the CeA was significantly increased after force application, suggesting that TRPV1 in the CeA may be involved in the regulation of tooth movement pain in rats.

Declarations

Author Contribution

Conceptualization, H.Q, R.W; methodology, H.Q, R.W and D.F; investigation, H.Q, D.F, S.L, F.J, Y.G and X.L; manuscript writing, H.Q, R.W; funding acquisition, H.Q; resources, H.Q; supervision, H.Q.

Data availability statement

Data that support the findings of this study and custom code used to analyse data are available from the corresponding author upon reasonable request.

Ethics Statement Animal care, handling, and all experiments were performed according to the guidelines of the National Institutes of Health of the United States and approved by the ethics committee of Xi`an Jiaotong University.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

Acknowledgment This study was supported by National Natural Science Foundation of China (Program No. 82001087) and the Fundamental Research Funds for the Central Universities (xzy012019104).

References

1. Long, H., Y. Wang, F. Jian, L. Liao, X. Yang, and W. Lai (2016) Current advances in orthodontic pain. International journal of oral science 8: 67-75. https://doi.org/10.1038/ijos.2016.24
2. Ashkenazi, M., Y. Berlin-Broner, and L. Levin (2012) Pain prevention and management during orthodontic treatment as perceived by patients. Orthodontics : the art and practice of dentofacial enhancement 13: e76-81.
3. Baldini, A., A. Nota, C. Santariello, V. Assi, F. Ballanti, and P. Cozza (2015) Influence of activation protocol on perceived pain during rapid maxillary expansion. The Angle orthodontist 85: 1015-20. https://doi.org/10.2319/112114-833.1
4. Panda, S., V. Verma, A. Sachan, and K. Singh (2015) Perception of pain due to various orthodontic procedures. Quintessence international (Berlin, Germany : 1985) 46: 603-9. https://doi.org/10.3290/j.qi.a33933
5. Byers, M. (1985) Sensory innervation of periodontal ligament of rat molars consists of unencapsulated Ruffini-like mechanoreceptors and free nerve endings. The Journal of comparative neurology 231: 500-18. https://doi.org/10.1002/cne.902310408

6. Yang, Z., Y. Wang, W. Luo, X. Hua, P. Wamalwa, J. Wang, Z. Zhao, Y. Lu, Z. Liao, and W. Lai (2009) Trigeminal expression of N-methyl-D-aspartate receptor subunit 1 and behavior responses to experimental tooth movement in rats. The Angle orthodontist 79: 951-7. https://doi.org/10.2319/082308-444.1
7. Simons, L., E. Moulton, C. Linnman, E. Carpino, L. Becerra, and D. Borsook (2014) The human amygdala and pain: evidence from neuroimaging. Human brain mapping 35: 527-38. https://doi.org/10.1002/hbm.22199
8. Carrasquillo, Y. and R. Gereau (2007) Activation of the extracellular signal-regulated kinase in the amygdala modulates pain perception. The Journal of neuroscience : the official journal of the Society for Neuroscience 27: 1543-51. https://doi.org/10.1523/jneurosci.3536-06.2007
9. Ikeda, R., Y. Takahashi, K. Inoue, and F. Kato (2007) NMDA receptor-independent synaptic plasticity in the central amygdala in the rat model of neuropathic pain. Pain 127: 161-72. https://doi.org/10.1016/j.pain.2006.09.003
10. Aggleton, J., S. Vann, C. Oswald, and M. Good (2000) Identifying cortical inputs to the rat hippocampus that subserve allocentric spatial processes: a simple problem with a complex answer. Hippocampus 10: 466-74. https://doi.org/10.1002/1098-1063(2000)10:4<466::Aid-hipo13>3.0.Co;2-y
11. Neugebauer, V., W. Li, G. Bird, and J. Han (2004) The amygdala and persistent pain. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry 10: 221-34. https://doi.org/10.1177/1073858403261077
12. Qiao, H., Y. Gao, Q. Huang, and R. Jia (2020) The central nucleus of the amygdala lesion attenuates orthodontic pain during experimental tooth movement in rats. Brain and behavior 10: e01506. https://doi.org/10.1002/brb3.1506
13. Cortright, D., M. Crandall, J. Sanchez, T. Zou, J. Krause, and G. White (2001) The tissue distribution and functional characterization of human VR1. Biochemical and biophysical research communications 281: 1183-9. https://doi.org/10.1006/bbrc.2001.4482
14. Roberts, J., J. Davis, and C. Benham (2004) [3H]Resiniferatoxin autoradiography in the CNS of wild-type and TRPV1 null mice defines TRPV1 (VR-1) protein distribution. Brain research 995: 176-83. https://doi.org/10.1016/j.brainres.2003.10.001
15. Sanchez, J., J. Krause, and D. Cortright (2001) The distribution and regulation of vanilloid receptor VR1 and VR1 5' splice variant RNA expression in rat. Neuroscience 107: 373-81. https://doi.org/10.1016/s0306-4522(01)00373-6
16. Szabo, T., T. Biro, A. Gonzalez, M. Palkovits, and P. Blumberg (2002) Pharmacological characterization of vanilloid receptor located in the brain. Brain research. Molecular brain research 98: 51-7. https://doi.org/10.1016/s0169-328x(01)00313-8
17. Jara-Oseguera, A., S. Simon, and T. Rosenbaum (2008) TRPV1: on the road to pain relief. Current molecular pharmacology 1: 255-69. https://doi.org/10.2174/1874467210801030255
18. Zimmermann, M. (1983) Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16: 109-110. https://doi.org/10.1016/0304-3959(83)90201-4

19. Kirschneck, C., P. Proff, J. Fanghaenel, M. Behr, U. Wahlmann, and P. Roemer (2013) Differentiated analysis of orthodontic tooth movement in rats with an improved rat model and three-dimensional imaging. Annals of anatomy = Anatomischer Anzeiger : official organ of the Anatomische Gesellschaft 195: 539-53. https://doi.org/10.1016/j.aanat.2013.08.003
20. Liao, L., H. Long, L. Zhang, H. Chen, Y. Zhou, N. Ye, and W. Lai (2014) Evaluation of pain in rats through facial expression following experimental tooth movement. European journal of oral sciences 122: 121-4. https://doi.org/10.1111/eos.12110
21. Lin, Y., A. Chou, H. Su, and K. Su (2020) Transient receptor potential V1 (TRPV1) modulates the therapeutic effects for comorbidity of pain and depression: The common molecular implication for electroacupuncture and omega-3 polyunsaturated fatty acids. Brain, behavior, and immunity 89: 604-614. https://doi.org/10.1016/j.bbi.2020.06.033
22. Hussain, A., M. Al Toubity, and W. Elias (2017) Methodologies in Orthodontic Pain Management: A Review. The open dentistry journal 11: 492-497. https://doi.org/10.2174/1874210601711010492
23. Larrea, M., R. Salvador, R. Cibrian, J. Gandia, and V. Paredes-Gallardo (2017) A New Equation for Predicting Evolution of Oral Pain in Orthodontic Treatment: A Longitudinal, Prospective Cohort Study. Journal of oral & facial pain and headache 31: 172-179. https://doi.org/10.11607/ofph.1723
24. Topolski, F., A. Moro, G. Correr, and S. Schimim (2018) Optimal management of orthodontic pain. Journal of pain research 11: 589-598. https://doi.org/10.2147/jpr.S127945
25. Yang, Z., W. Luo, J. Hou, Z. Zhao, F. Jian, P. Wamalwa, W. Lai, J. Wang, Y. Wang, and Z. Liao (2009) Development of a behavior model of pain induced by experimental tooth movement in rats. European journal of oral sciences 117: 380-4. https://doi.org/10.1111/j.1600-0722.2009.00639.x
26. Sotocinal, S., R. Sorge, A. Zaloum, A. Tuttle, L. Martin, J. Wieskopf, J. Mapplebeck, P. Wei, S. Zhan, S. Zhang, J. McDougall, O. King, and J. Mogil (2011) The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Molecular pain 7: 55. https://doi.org/10.1186/1744-8069-7-55
27. Liao, Z., H. Long, Z. Song, Y. He, and W. Lai (2021) Behavioral Responses and Expression of Nociceptin/Orphanin FQ and Its Receptor (N/OFQ-NOP System) during Experimental Tooth Movement in Rats. Pain research & management 2021: 9981732. https://doi.org/10.1155/2021/9981732
28. Singh, R., P. Jayaprakash, A. Yadav, M. Dawar, H. Grewal, and A. Mishra (2019) Evaluation of levels of Interleukin-1b, intensity of pain and tooth movement during canine retraction using different magnitudes of continuous orthodontic force. Journal of family medicine and primary care 8: 2373-2377. https://doi.org/10.4103/jfmpc.jfmpc_357_19
29. Will, L. (2016) Orthodontic Tooth Movement: A Historic Prospective. Frontiers of oral biology 18: 46-55. https://doi.org/10.1159/000351899
30. Veinante, P., I. Yalcin, and M. Barrot (2013) The amygdala between sensation and affect: a role in pain. Journal of molecular psychiatry 1: 9. https://doi.org/10.1186/2049-9256-1-9

31. Li, F. and F. Wang (2021) TRPV1 in Pain and Itch. Advances in experimental medicine and biology 1349: 249-273. https://doi.org/10.1007/978-981-16-4254-8_12

Figures

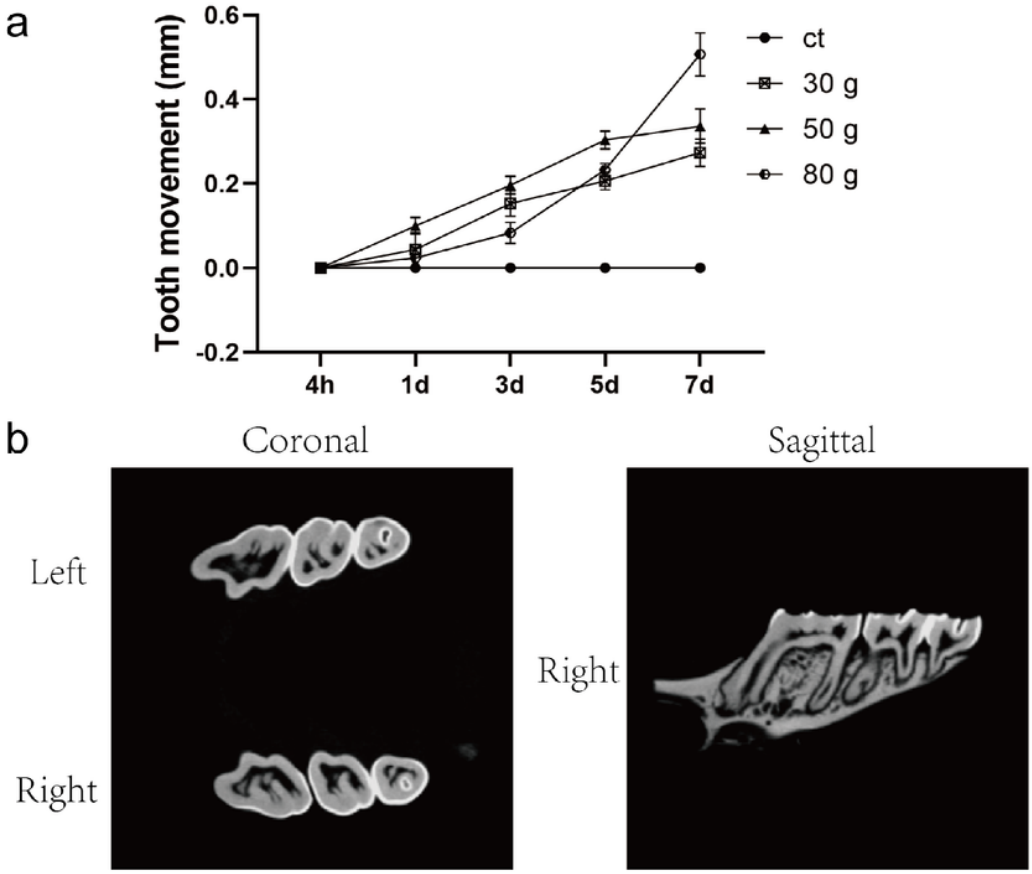

Figure 1

Micro-CT performance of EMT. (a) ETM rate in each group from 1 day to 7 d (mm / d). (b) ETM image of 50 g 7 d. Abbreviations: ETM, experimental tooth movement

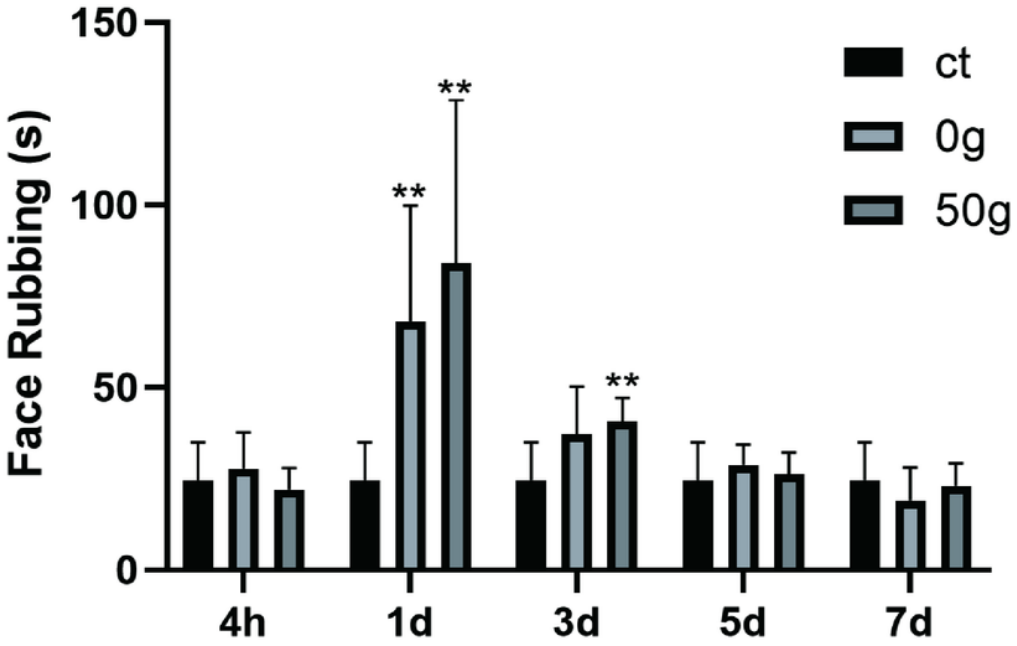

Figure 2

Face grooming behaviour following ETM. $^{**}P<0.01$

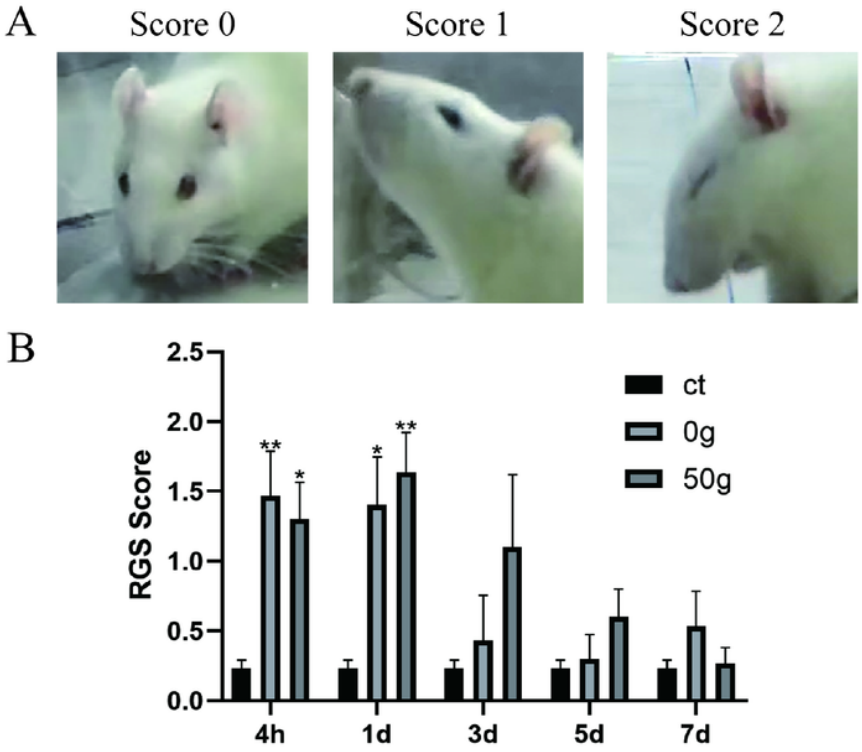

Figure 3

Rat grimace scale (RGS) score following ETM. (a) RGS coding magnitude. (b) Quantitative analysis. * $P < 0.05$, ** $P < 0.01$

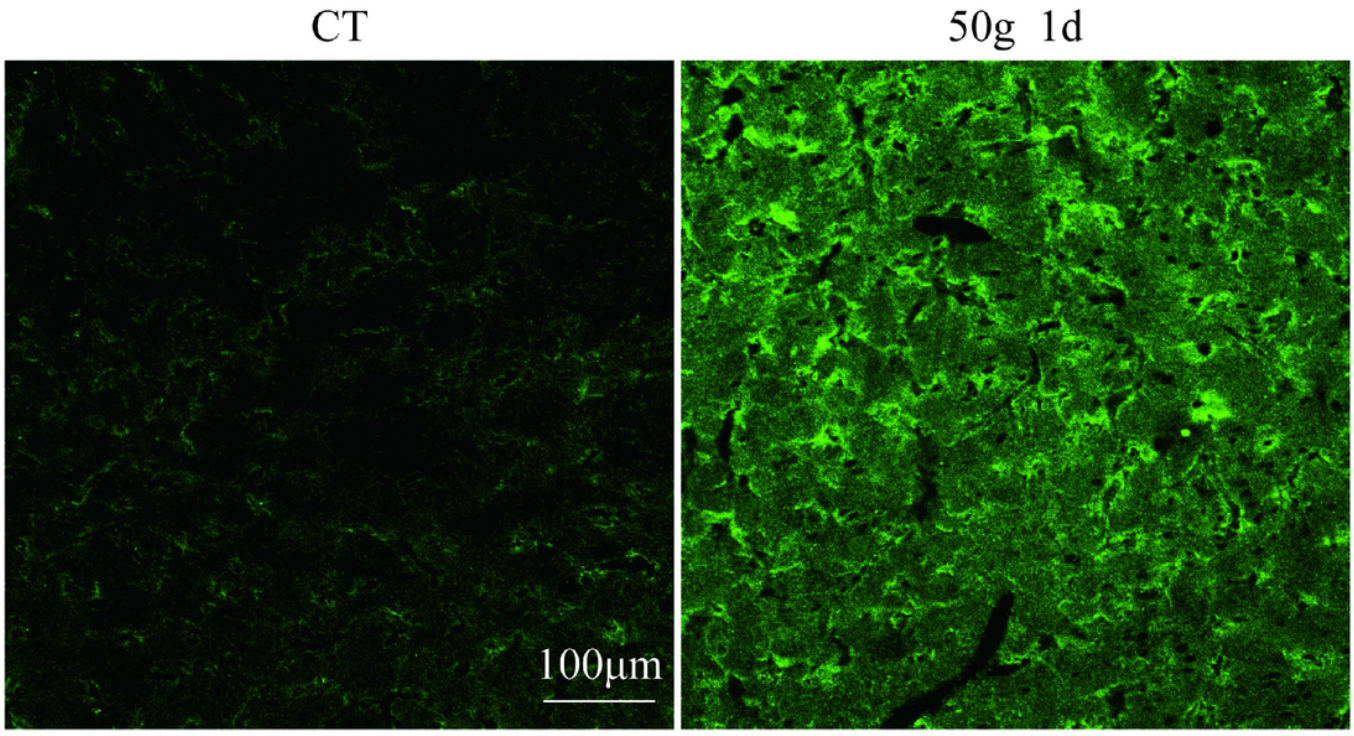

Figure 4

Immunofluorescent expression of TRPV1 in the CeA. Abbreviations: TRPV1, Transient receptor potential vanilloid 1; CeA, central nucleus of amygdala. TRPV1-immunoreactive neurons are labeled green. Scale bar = 100 μm

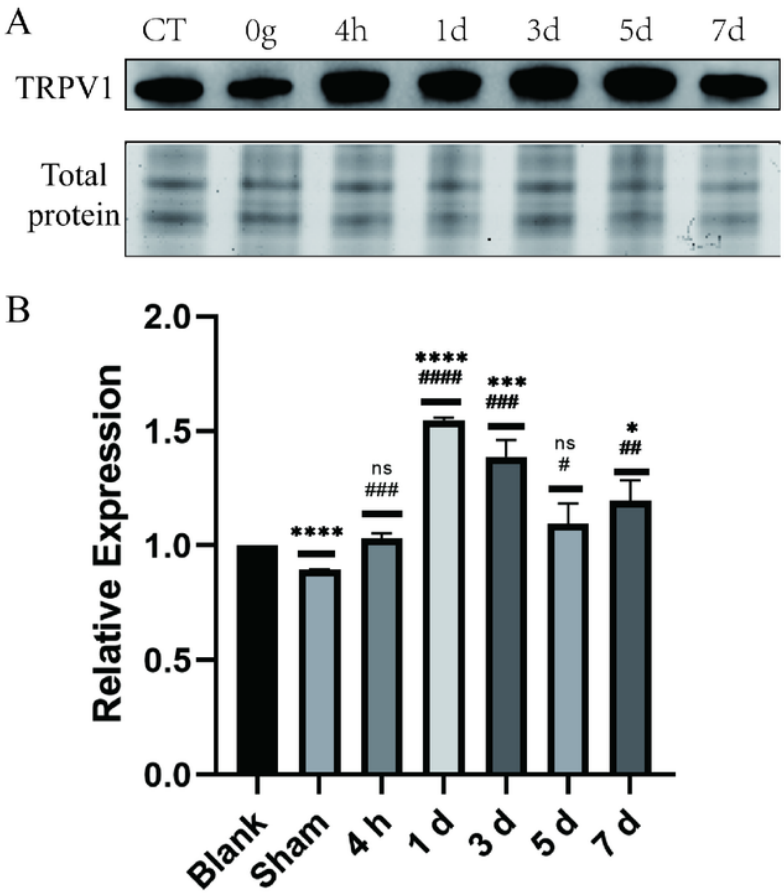

Figure 5

Western blots of TRPV1 in the CeA. (a) TRPV1 expressed in the CeA. (b) Quantitative analysis. ns, no significance. * P< 0.05, *** P <0.001, **** P <0.01, compared with Blank Group; P < 0.05, P <0.01, P <0.001, P <0.0001, compared with Sham Group

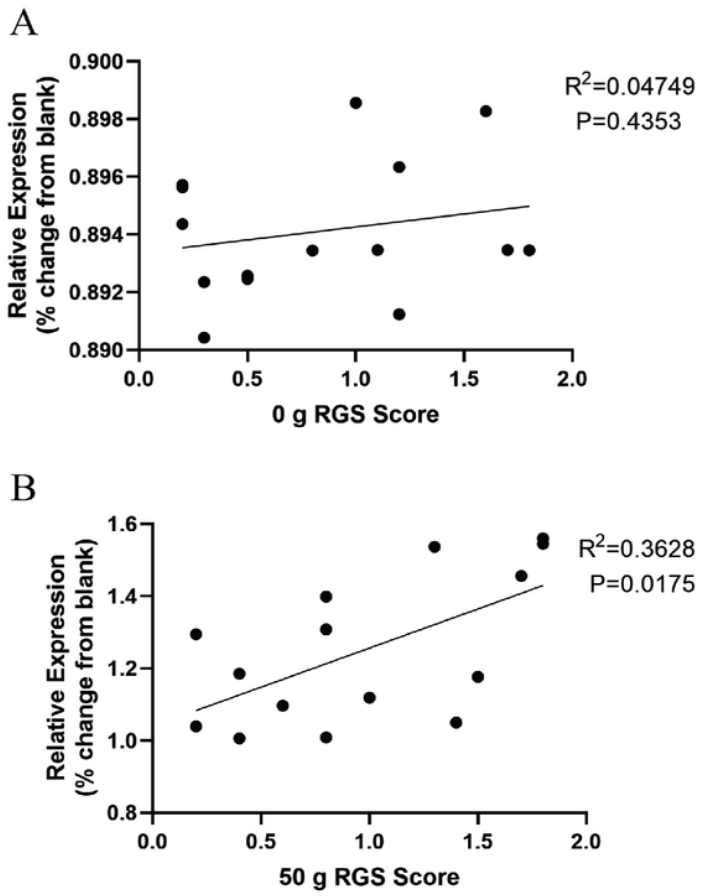

Figure 6

Correlation between the time spent on RGS and expression of TRPV1 in the CeA. Pearson correlation revealed significant correlation between the intensity of TRPV1 and the RGS score in sham-treated (a) (R^2 = 0.04749, *P* 0.05) and experimental (b) (R^2 = 0.3628, *P* < 0.05) groups