Effect of Vanillin on Emotional Stress and Pain-Related Behaviours Induced by Experimental Tooth Movement in Rats

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Research Article

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Abstract

Background

Pain is a common symptom of orthodontic treatment. Therefore, this study aimed to determine the effect of vanillin on emotional stress and pain-related behaviours induced during tooth movement by observing the behaviours of orthodontic rats and to provide a therapeutic basis for the discomfort caused by orthodontic treatment.

Methods

A 75 g orthodontic force was applied via an orthodontic appliance to the left maxillary incisors and first molars of 200–250 g male Sprague-Dawley rats. The rats were then administered vanillin in three different ways on days 1, 2, 3, 5, and 7 (via gavage, sniffing, or coating around the gingiva of the orthodontic teeth). Behaviour was evaluated at each time point in an open field based on the number of lines crossed in the first 30 s and 5 min, the number of lines crossed to the centre, the rearing time, the facial grooming time, and the Resistance-to-Capture scores.

Results

In the experimental orthodontic stimulation, the rats showed anxious, pain-related behaviours such as nervousness, rearing, decreased interest in exploring their surroundings, and reduced mobility. Conversely, in orthodontic rats administered vanillin, anxiety and pain-related behaviours were alleviated, and their mobility was restored. Furthermore, each administration method has the advantages of reducing pain and increasing activity.

Conclusion

This study revealed that three vanillin administration methods have advantages in alleviating anxiety and pain during experimental tooth movement, which can serve as a reference for clinicians to treat patients with orthodontic pain.

Background

Pain is a common symptom of orthodontic treatment, with a reported incidence of 72–100% among orthodontic patients who experience various degrees of pain [1]. This pain typically occurs within a few hours to a day after the treatment, peaks on day 3, and returns to baseline on day 14 [2, 3]. The application of orthodontic force rebuilds periodontal tissue and triggers the release of various inflammatory mediators such as Prostaglandin E2 (PEG2), inducible nitric oxide synthase (iNOS), bradykinin, 5-

Hydroxytryptamine (5-HT), and substance P [4]. These mediators activate the injury receptors and transmit pain signals. These pain signals are then transmitted via the Aδ and C fibres of the trigeminal nerve to the trigeminal ganglion cytosol, converted by neurons at all levels, before being projected to the cerebral cortex, leading to the perception of nociception [1]. However, while reducing local inflammation can be an effective method of pain relief, concerns regarding discomfort during the initial phase of orthodontics and the psychosocial implications of wearing braces can cause high levels of anxiety [5]. In particular, dental anxiety may cause negative thoughts, fears, sleep disturbances [6], interference with work and personal relationships [7–10], and even affect compliance with orthodontic treatment [11]. Some studies have compared the emotional status of patients before and after orthodontic treatment and, in many cases, showed a positive association between pain and stress [12, 13]. In addition, during dental injections, anxious patients tend to experience more pain than less anxious patients [14], as dental anxiety consistently influences the perception of pain throughout various dental procedures, thereby highlighting the importance of anxiety management for highly anxious patients and pain control for all dental patients [15, 16]. Consequently, psychological interventions have been suggested as a means to alleviate pain.

Many methods, such as musical intervention, verbal and written advice from doctors, and visual activation, have been used to demonstrate pain perception in patients undergoing dental treatment [17–21]. Another notable method is olfaction, which plays an important role in transmitting and processing noxious stimuli. A prior study reported that smell and taste appear to interact at both the behavioural and neural levels, share common features, and are strongly linked to emotion and cognition [22]. Although there is evidence that olfactory and gustatory substances have powerful effects on experimental pain, such as unpleasant chemosensory stimuli, which increase the perception of pain in patients with chronic oral burns [23], other studies have reported that olfactory input stimulated by linalool odour has an analgesic effect [24, 25]. Currently, nonsteroidal anti-inflammatory drugs (NSAIDs) are most commonly used to relieve pain because they block the formation of arachidonic acid during the prostaglandin production cycle [26]. However, patients often worry about the side effects of drugs. Moreover, because procedures such as electrical and vibration stimulations are more complex, researchers have been trying to find a safe and convenient method to relieve orthodontic pain.

Vanillin, an aromatic compound extracted from vanilla beans, has a strong milk fragrance and stable aroma and is widely used as a flavouring agent or food additive. Recently, the pharmacological activities of vanillin have attracted increasing attention. As a natural small-molecule substance, vanillin can quickly pass through the blood-brain barrier compared to plasma and exhibit greater stability in the brain tissue [27]. Vanillin has also been reported to exhibit anti-inflammatory [28–31], antinociceptive [32] and anti-stress activities, as studies have shown that vanillin elicits a protective effect in rats subjected to chronic mild stress [33], and the antinociceptive potential of vanillin has been demonstrated in acetic acid-induced visceral inflammatory pain models [32]. However, its application in orthodontics is yet to be explored.

Therefore, this study aimed to investigate the role of vanillin in relieving the emotional stress and pain induced by experimental tooth movement (ETM) in rats as well as provide a basis to enhance the comfort and experience of patients undergoing orthodontic treatment.

Methods

Animals

A total of 40 male Sprague–Dawley rats weighing between 200 and 250 g were used in this study. The rats were housed in clear plastic cages containing soft bedding in a colony room following a 12-hour light-dark cycle with an ambient temperature maintained at 21–23°C. Food and water were randomly provided, and the feeding and tests were conducted in a room with a background noise level of 40–50 dB. Before the commencement of the study, the rats were allowed to acclimatise to the housing conditions for 5 days. All possible measures were taken to minimise animal suffering and the number of animals used in this study.

Experimental Design

Animals were randomly divided into four orthodontic groups and one control group (eight rats per group[32]) (Fig. 1A). A 75 g orthodontic force was applied to the left maxillary incisor and first molars using a fixed nickel-titanium (Ni-Ti) alloy closed-coil spring in the orthodontics groups [35]. The groups were as follows: Group O received orthodontics without any drugs, Group O + iV received 50 mg/kg of vanillin[26] intragastrically (1% w/v diluted in 25°C purified water, i.g.) (Fig. 1B), Group O + sV received aroma of 1% w/v vanillin (vanillin solution was added to cotton balls and placed at the bottom of the aromatherapy barrel [36]) with the rats placed on a perforated platform in the middle of the aromatherapy barrel, one per barrel (Fig. 1C), Group O + cV received 1% w/v vanillin coated around the gingiva of the orthodontic teeth (Fig. 1D) and Group N received no appliance or drug. Vanillin was administered for 7 days. After administering vanillin for 30 min, the rats underwent behavioural tests on days 1, 2, 3, 5, and 7. To assess the whole-body effects, the animals were weighed before treatment and at regular intervals during the experimental period.

Behavioural Testing

Two behavioural tests were performed: an Open-Field Test and the Resistance-to-Capture Test [37]. All tests were conducted during the light phase of the light/dark cycle and were performed on days 1, 2, 3, 5, and 7 after the orthodontic appliances were placed. Each rat was brought to the test room for behavioural testing and promptly returned to the colony room afterward. The test room was quiet and temperature controlled (22°C). The rats were recorded using a camera placed 2 m above the testing box during the tests, and the images taken were then analysed by five observers blinded to the grouping of the animals.

Open-Field Test

During this test, each rat was placed in one corner of a 100 × 100 cm open field surrounded by 40 cm high plastic board walls. The floor was composed of an odourless plastic board divided into 25 squares of equal size. The behaviour of the rat in the open field was recorded for 5 min. Then the following parameters were analysed: (1) the number of lines crossed in the first 30 s, (2) the total number of lines crossed in 5 min, (3) the number of lines crossed to the centre of the open field, (4) the rearing time, and (5) the facial grooming time. A line was considered crossed when all four paws crossed the line. Facial grooming time was measured using a stopwatch.

Resistance-to-Capture Test

Following the open field test, a resistance-to-capture test was performed. The test involved measuring the animal's resistance to being picked up by the examiner. The level of resistance was evaluated as follows: 0, easy to pick up; 1, vocalises or shies away from hand; 2, shies away from hand and vocalises; 3, runs away from hand; 4, runs away and vocalises; 5, bites or attempts to bite; and 6, launches a jump attack.

Measuring tooth movement distance

After 7 days of orthodontic treatment, the rats were anaesthetised. The maxillary region of the rats was then carefully separated and fully exposed for further analysis. With the aid of a vernier calliper (Fig. 2A), the distance between the left maxillary first molar and second molar was measured three times by three researchers.

Statistical analysis

Statistical analyses were performed using SPSS (version 26; IBM Corp, Armonk, NY, USA) and GraphPad Prism (version 6.01; GraphPad Software, San Diego, Calif, USA). The Shapiro-Wilk test revealed that the data were normally distributed ($p < 0.05$). A repeated-measures analysis of variance and Dunnett's post-hoc comparison tests were conducted, with statistical significance set at $p < 0.05$ to analyse the changes in parameters at different time intervals.

Results

Effects of vanillin on the tooth movement distance of rats during ETM

Before studying the effect of vanillin on the pain and anxiety caused by orthodontic treatment, it was necessary to determine its role in tooth movement during ETM. The average distance of the first molar movement measured on the 7th day in each group is shown in Fig. 2B and Tab. 1. The distance between the first and second molars in all ETM groups was statistically significant compared to the N group ($p < 0.05$). However, there was no significant difference in the distance between the vanillin-treated groups ($p > 0.05$). This suggests that the application of vanillin did not affect tooth movement.

Furthermore, we found that the body weight of the rats in the N group increased gradually from 227.8 ± 0.7 g on day 0 to 252.0 ± 2.0 g on day 7, and compared with the body weight on day 0, that on day 7 increased significantly ($p < 0.001$) (Tab. 2). However, for rats subjected to 75 g of orthodontic force, the weight decreased in the first 2 days before increasing after the third day (Fig. 3). In group O, the mean body weight of the rats decreased significantly from 227.6 ± 2.4 g before orthodontics to 221.8 ± 2.8 g on day 2 ($p < 0.05$). After that, the body weight steadily increased, reaching 238.8 ± 3.7 g on day 7. Likewise, the body weights of rats administered vanillin showed a similar trend to group O but with a faster growth rate (Tab. 2 and Fig. 3). These findings suggest that the use of vanillin in vivo may be safe, or potentially less toxic, and may alleviate discomfort and promote normal feeding in rats.

Vanillin relieves emotional stress and pain during ETM in rats

After confirming the safety and effect of vanillin on tooth movement, it was necessary to determine the role of vanillin in emotional stress and pain-related behaviours induced by ETM in Rats. First, an Open-Field Test was conducted (Fig. 4A). In this experiment, the number of lines crossed in the open field represented the mobility of the rats and was analysed at each time point (days 1, 2, 3, 5, and 7) in the five groups (Figs. 4B-C). During the first 30 s, the number of lines crossed was lower than that observed at 5 min. The number of lines crossed decreased in the first 2 days and then increased after the third day in the O and O + iV groups. However, different trends were observed in the O+sV and O + cV groups, as they increased during the first 2 days (compared to the O and O + iV groups), peaked on day 2, and then decreased. Afterward, the lines crossed by the O+sV group gradually increased and returned to the group N values on day 7. In contrast, lines crossed by the O+cV group increased on day 5 and then decreased on day 7, below the level observed in the other groups, but showed no statistical significance. Analysis of the number of lines crossed into the central area showed that rats in the vanillin group entered the central area more often than those in groups N and O (Fig. 4D). The number of rearing time in the open field of group O rats was lower compared to the vanillin-treated group (Fig. 4E), and statistically significant differences were observed on day 2 (Fig. 4E: day 2 N group vs O group, $p < 0.05$; O+sV group vs O group, $p < 0.05$; O+cV group vs O group, $p < 0.05$). Facial grooming time in the O and vanillin groups increased in the first 2 – 3 days, reaching a peak (Fig. 4F: day 1: N group vs O group $p < 0.05$; day 2: N group vs O group $p < 0.01$; day 3: N group vs O group $p < 0.01$), and then decreased to the same level as that of the N group on day 7. Notably, the facial grooming time of the vanillin group was shorter than those in group O (Fig. 4F: day 1 O+iV group vs O group $p < 0.01$; day 1: O+cV group vs O group $p < 0.01$; day 2: O+cV group vs O group $p < 0.01$; day 5: O+cV group vs O group $p < 0.05$; day 7: O+cV group vs O group $p < 0.01$). The resistance-to-capture ability indicates resistance to adverse external stimuli (Fig. 5A), and it decreased sharply in the O group after applying 75 g of orthodontic force during the first 3 days (Fig. 5B) before gradually increasing after day 3. Rats treated with vanillin showed a similar trend; however, the O+sV and O+cV groups showed faster recovery when resisting capture (Fig. 5B). These results demonstrate that after orthodontics were applied, the rats' mobility weakened; however, the administration of vanillin in all three vanillin treatment groups increased their mobility, improved exploration of the external environment, and decreased grooming behaviour after orthodontics.

Discussion

Changes in the occlusal contact and compressed periodontal ligament can lead to discomfort and pain in orthodontic patients. Aksoy et al. [38] demonstrated that orthodontic treatment affects patients' anxiety and cortisol levels. Similarly, studies have reported that the application of orthodontic appliances reduced oral health-related quality of life and mental health conditions during the first month of treatment, even though oral health-related quality of life and psychological status recovered after the orthodontic treatment [39, 40]. Reduced activity and interest in exploring one's surroundings in an open field are considered indicators of anxiety and fear in unknown areas [41]. In addition, consistent with our findings, several studies in both humans [19, 42, 43] and experimental animals [34, 44, 45] agree that pain associated with ETM peaks at 1–3 days (as we observed that ETM led to a remarkable increase in facial grooming behaviour from day 1 to day 3 in the O group). Thus, this means that orthodontic treatment not only deals with periodontal pain but also psychological changes, such as anxiety and depression. Therefore, we hypothesized that there is an association between depression-and anxiety-like symptoms and ETM in rats based on their behavioural performance. To reduce the discomfort associated with orthodontics, a meta-analysis [46] revealed differences in pain between orthodontic patients treated with laser therapy and those treated with a placebo at 24 and 72 h. Likewise, Kaya et al. [47] proved that ibuprofen is often used to reduce pain because it reduces IL-1β and substance P levels in the gingival crevicular fluid. However, because patients often worry about the side effects of taking drugs and electrical and vibration stimulations are more complex to perform, researchers have tried to find a safe and acceptable method to relieve orthodontic pain. Notably, in this study, two non-invasive methods, sniffing and coating, were used to examine the inhibitory effect of vanillin on orthodontic pain, and our results showed a significant reduction in grooming time in the O+sV and O+cV groups on days 5 and 7, indicating that pain was alleviated by these methods.

Vanillin is a natural compound with anti-inflammatory properties. A prior study depicting a model of mechanical injury [48] showed that vanillin reduced IL-6, IL-8, COX-2, iNOS expression, and NO release, indicating a potential therapeutic effect of vanillin as an inflammatory modulator in tissue repair and regeneration. Another study also reveals that vanillin decreases the levels of TNF-α, IL-6, IL-1β, and IFN-α in gastric ulcers, promotes mucus production, and preserves gastric mucosa integrity [49]. However, studies on the applications of vanillin in orthodontic treatment are lacking. In this study, rats treated with vanillin showed higher mobility, more interest in exploring the external environment, and less grooming behaviour than those in group O, suggesting that vanillin relieves orthodontic pain. In addition, we explored the effect of vanillin's fragrance on orthodontic pain by permeating the blood-brain barrier, and the results show that rats in the O+sV group exhibited decreased grooming behaviour after sniffing vanillin. Thus, these findings suggest that vanillin has a relieving effect on orthodontic pain and promotes emotional calmness. However, there is no clear conclusion regarding the effectiveness of these three administration methods. For example, in the first 2 days, the O+sV and O + cV groups boosted the rats' mobility, which was then followed by a decrease, with no abnormal changes in body weight or health status. In future studies, we will continue to explore the method of administration that is most effective in relieving orthodontic pain and anxiety. Moreover, vanillin pharmacological actions and mechanisms of action still require further study. Our study provides a novel approach for alleviating orthodontic pain.

First, patients with orthodontic pain often refuse to take painkillers because they are worried about their side effects. Therefore, considering that vanillin is commonly used as a food additive, its acceptance may increase if used to alleviate orthodontic pain and anxiety. Second, our experiment used three methods of administration: intragastric, sniffing, and coating, all of which have the effect of relieving orthodontic pain and reducing emotional stress. We have innovatively explored the effect of smell on orthodontic pain via sniffing of vanillin, which we believe has provided ideas for the development of clinical drug dosage forms in the future, such as vanillin that can be made into edible tablets, sniffing spray, or locally coated gel.

Conclusions

This study shows that vanillin administration restored depression-and anxiety-like behaviours induced by ETM; however, further research is needed to investigate whether vanillin can serve as a therapeutic option for depression-and anxiety-like symptoms in clinical orthodontic treatments.

Abbreviations

ETM: Experimental tooth movement

PEG2: Prostaglandin E2

iNOS: inducible nitric oxide synthase

5-HT: 5-Hydroxytryptamine

NSAIDs: nonsteroidal anti-inflammatory drugs

COX-2: Cyclooxygenase-2

TNF-α: Tumor necrosis factor-α

IL-6: interleukin 6

IL-8: interleukin 8

IFN-α: Interferon α

N group: Nonoperative group

O group: Orthodontics group

O+iV group: Orthodontics+ intragastrical administrated of Vanillin group

O+sV group: Orthodontics+ sniffing Vanillin group

O+cV group: Orthodontics+ coating Vanillin group

Declarations

Ethics approval and consent to participate

The experimental procedures and the animal use and care protocols were approved by the Institutional Animal Care and Use Committee of Anhui Medical University (Permit number: LLSC20221085). All procedures were performed by the Regulations of the Animal Management Regulations and Administrative Measures on Experimental Animal. All studies involving animals were reported in accordance with the ARRIVE guidelines for reporting experiments involving animals.

Consent for publication

There are no contradictions in publishing the work.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available but are available from the corresponding author upon reasonable request.

Competing interests

There are no contradictions in publishing the work.

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Authors’ contributions

Shiyu Jin contributed to the design of the work, data interpretation and original draft preparation; Qunyan Zhang contributed to the design of the work and data interpretation; Siqi Ding contributed to supervision; Chuan Wu contributed to data analysis; Xiuyun Zheng contributed to data analysis; Chunfeng Fu contributed to data analysis; Quancheng Han contributed to data analysis; Xiaoyu Liu contributed to manuscript review and editing; Wenning Wu contributed to manuscript review and editing; and Tingting Wu conceptualization, funding acquisition, and manuscript review and editing.

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References

1. Long, H., et al., *Current advances in orthodontic pain.* Int J Oral Sci, 2016. **8**(2): p. 67-75.
2. Guo, R., et al., *Transient receptor potential Vanilloid 1-based gene therapy alleviates orthodontic pain in rats.* Int J Oral Sci, 2019. **11**(1): p. 11.
3. Wu, C., et al., *Response of human periodontal ligament to orthodontic force using superb microvascular imaging.* Am J Orthod Dentofacial Orthop, 2022.
4. Li, Y., et al., *Orthodontic tooth movement: The biology and clinical implications.* Kaohsiung J Med Sci, 2018. **34**(4): p. 207-214.
5. Sari, Z., et al., *Does orthodontic treatment affect patients' and parents' anxiety levels?* European Journal of Orthodontics, 2005. **27**(2): p. 155-159.
6. Qiao, H., et al., *The central nucleus of the amygdala lesion attenuates orthodontic pain during experimental tooth movement in rats.* Brain Behav, 2020. **10**(1): p. e01506.
7. Cohen, S.M., J. Fiske, and J.T. Newton, *The impact of dental anxiety on daily living.* Br Dent J, 2000. **189**(7): p. 385-90.
8. Yildirim, E. and S. Karacay, *Evaluation of anxiety level changes during the first three months of orthodontic treatment.* Korean J Orthod, 2012. **42**(4): p. 201-6.
9. Lin, W., et al., *Factors associated with orthodontic pain.* J Oral Rehabil, 2021. **48**(1365-2842 (Electronic)): p. 1135-1143.
10. Cao, Y., *Occlusal disharmony and chronic oro-facial pain: from clinical observation to animal study.* J Oral Rehabil, 2022. **49**(2): p. 116-124.
11. Zhang, F., et al., *Altered Brain Topological Property Associated With Anxiety in Experimental Orthodontic Pain.* Front Neurosci, 2022. **16**(1662-4548 (Print)): p. 907216.
12. Fried, K.H., *Emotional stress during retention and its effect on tooth position.* Angle Orthod, 1976. **46**(1): p. 77-85.
13. Lin, F., et al., *Individualized intervention to reduce anxiety in adult orthodontic patients based on Q methodology.* Am J Orthod Dentofacial Orthop, 2017. **152**(2): p. 161-170.
14. van Wijk, A.J. and J. Hoogstraten, *Anxiety and pain during dental injections.* J Dent, 2009. **37**(9): p. 700-4.
15. Weisenberg, M., et al., *Relevant and irrelevant anxiety in the reaction to pain.* Pain, 1984. **20**(4): p. 371-383.
16. Lin, C.S., S.Y. Wu, and C.A. Yi, *Association between Anxiety and Pain in Dental Treatment: A Systematic Review and Meta-analysis.* Journal of Dental Research, 2017. **96**(2): p. 153-162.
17. Bertacco, M., et al., *Effect of personalized musical intervention on burden of care in dental implant surgery: A pilot randomized controlled trial.* J Dent, 2022. **120**: p. 104091.
18. Di Nasso, L., et al., *Influences of 432 Hz Music on the Perception of Anxiety during Endodontic Treatment: A Randomized Controlled Clinical Trial.* J Endod, 2016. **42**(9): p. 1338-43.

19. Montebugnoli, F., et al., *Effect of verbal and written information on pain perception in patients undergoing fixed orthodontic treatment: a randomized controlled trial.* Eur J Orthod, 2020. **42**(5): p. 494-499.
20. Tang, Y.L., et al., *Green light analgesia in mice is mediated by visual activation of enkephalinergic neurons in the ventrolateral geniculate nucleus.* Sci Transl Med, 2022. **14**(674): p. eabq6474.
21. Longo, M.R., et al., *Linking pain and the body: neural correlates of visually induced analgesia.* J Neurosci, 2012. **32**(8): p. 2601-7.
22. Sandri, A., et al., *Pain, Smell, and Taste in Adults: A Narrative Review of Multisensory Perception and Interaction.* Pain Ther, 2021. **10**(1): p. 245-268.
23. Sandri, A., et al., *Unpleasant olfactory and gustatory stimuli increase pain unpleasantness in patients with chronic oral burning pain: An exploratory study.* Eur J Pain, 2022. **26**(5): p. 1094-1106.
24. Tashiro, S., et al., *Odour-induced analgesia mediated by hypothalamic orexin neurons in mice.* Sci Rep, 2016. **6**: p. 37129.
25. Alkanan, S.A.M., et al., *Dental pain perception and emotional changes: on the relationship between dental anxiety and olfaction.* BMC Oral Health, 2023. **23**(1): p. 175.
26. Beaudry, F., et al., *Pharmacokinetics of vanillin and its effects on mechanical hypersensitivity in a rat model of neuropathic pain.* Phytother Res, 2010. **24**(4): p. 525-30.
27. Ueno, H., et al., *Comprehensive behavioral study of the effects of vanillin inhalation in mice.* Biomed Pharmacother, 2019. **115**: p. 108879.
28. Kim, M.E., et al., *Anti-Neuroinflammatory Effects of Vanillin Through the Regulation of Inflammatory Factors and NF-κB Signaling in LPS-Stimulated Microglia.* Appl Biochem Biotechnol, 2019. **187**(3): p. 884-893.
29. Ghanim, A.M.H., N.S. Younis, and H.A. Metwaly, *Vanillin augments liver regeneration effectively in Thioacetamide induced liver fibrosis rat model.* Life Sci, 2021. **286**: p. 120036.
30. Guo, W., et al., *Vanillin protects the blood-milk barrier and inhibits the inflammatory response in LPS-induced mastitis in mice.* Toxicol Appl Pharmacol, 2019. **365**: p. 9-18.
31. Chen, H., J. Zheng, and J. Ma, *Vanillin ameliorates changes in HIF-1α expression and neuronal apoptosis in a rat model of spinal cord injury.* Restor Neurol Neurosci, 2019. **37**(1): p. 21-29.
32. Park, S.H., et al., *Antinociceptive profiles and mechanisms of orally administered vanillin in the mice.* Arch Pharm Res, 2009. **32**(11): p. 1643-9.
33. Abo-youssef, A.M., *Possible antidepressant effects of vanillin against experimentally induced chronic mild stress in rats.* Beni-Suef University Journal of Basic and Applied Sciences, 2016. **5**(2): p. 187-192.
34. Yang, Z., et al., *Development of a behavior model of pain induced by experimental tooth movement in rats.* Eur J Oral Sci, 2009. **117**(4): p. 380-4.
35. Yang, H., et al., *Acid-improved DNAzyme-based chemiluminescence miRNA assay coupled with enzyme-free concatenated DNA circuit.* Biosens Bioelectron, 2022. **204**: p. 114060.

36. Xu, J., et al., *Vanillin-induced amelioration of depression-like behaviors in rats by modulating monoamine neurotransmitters in the brain.* Psychiatry Res, 2015. **225**(3): p. 509-14.
37. Shibazaki, T., et al., *Effect of celecoxib on emotional stress and pain-related behaviors evoked by experimental tooth movement in the rat.* Angle Orthod, 2009. **79**(6): p. 1169-74.
38. Aksoy, A., et al., *Assessment of Pain, Anxiety, and Cortisol Levels During the Initial Aligning Phase of Fixed Orthodontic Treatment.* Turk J Orthod, 2019. **32**(1): p. 34-40.
39. Johal, A., et al., *The impact of orthodontic treatment on quality of life and self-esteem in adult patients.* Eur J Orthod, 2015. **37**(3): p. 233-7.
40. Abreu, L.G., et al., *Impact of orthodontic treatment on adolescents' quality of life: a longitudinal evaluation of treated and untreated individuals.* Quality of Life Research, 2018. **27**(8): p. 2019-2026.
41. Sturman, O., P.L. Germain, and J. Bohacek, *Exploratory rearing: a context- and stress-sensitive behavior recorded in the open-field test.* Stress, 2018. **21**(5): p. 443-452.
42. Ogura, M., et al., *Pain intensity during the first 7 days following the application of light and heavy continuous forces.* Eur J Orthod, 2009. **31**(3): p. 314-9.
43. Kavaliauskiene, A., et al., *Pain and discomfort perception among patients undergoing orthodontic treatment: results from one month follow-up study.* Stomatologija, 2012. **14**(4): p. 118-25.
44. Sood, M., P. Bhatt, and B.J. Sessle, *Mechanical and thermal hypersensitivities associated with orthodontic tooth movement: a behavioral rat model for orthodontic tooth movement-induced pain.* J Oral Facial Pain Headache, 2015. **29**(1): p. 60-9.
45. Liao, L.N., et al., *Evaluation of pain in rats through facial expression following experimental tooth movement.* European Journal of Oral Sciences, 2014. **122**(2): p. 121-124.
46. Deana, N.F., et al., *Effectiveness of Low-Level Laser Therapy in Reducing Orthodontic Pain: A Systematic Review and Meta-Analysis.* Pain Res Manag, 2017. **2017**: p. 8560652.
47. Kaya, Y., et al., *Effects of ibuprofen and low-level laser therapy on orthodontic pain by means of the analysis of interleukin 1-beta and substance P levels in the gingival crevicular fluid.* J Orofac Orthop, 2021. **82**(3): p. 143-152.
48. Costantini, E., et al., *Assessment of the Vanillin Anti-Inflammatory and Regenerative Potentials in Inflamed Primary Human Gingival Fibroblast.* Mediators Inflamm, 2021. **2021**: p. 5562340.
49. Ciciliato, M.P., et al., *Anti-Inflammatory Effect of Vanillin Protects the Stomach against Ulcer Formation.* Pharmaceutics, 2022. **14**(4).

Tables

Table 1. Tooth movement distances of rats in various groups on the 7th day. (n=8, x±SEM, l/mm)

Group	Tooth movement distance
Control	
Nonoperation group	0
Nonmedication group	0.27±0.06
Vanillin (1% w/v)	
Intragastric group	0.24±0.08
Sniffing group	0.32±0.15
Coating group	0.26±0.09

Tooth movement distance. Distance between the maxillary first molar and maxillary second molar was measured by 0.02 accuracy vernier calipers after 7 days orthodontics. Comparison with the O group. Error bars indicate SEM. *p<0.05.

Table 2. Weight of rats during orthodontics

Rat weight, g	d0	d1	d2	d3	d5	d7
N	227.8±0.7	232.3±1.0	237.1±1.2***	243.9±1.1***	248.4±1.8***	252.0±2.0***
O	227.6±2.4	222.6±2.9	221.8±2.8*	224.4±2.2	232.6±2.9	238.8±3.7***
O+i V	227.8±1.7	226.4±1.4	222.5±1.4	228.3±1.7	239.8±1.9***	245.9±1.7***
O+sV	228.3±0.9	223.9±2.6	226.3±1.7	230.6±2.1	237.6±1.8***	246.8±2.4***
O+cV	228.1±1.0	226.4±1.4	222.5±1.4	228.3±1.7	239.8±1.9***	245.9±1.7***

Weight. The mean value of body weights during the orthodontics of the 5 groups (mean ± SEM, n= 8). Comparison with the d0 group (before orthodontics); *P< 0.05, **P< 0.01, ***P< 0.001. SEM standard error of mean.

Figures

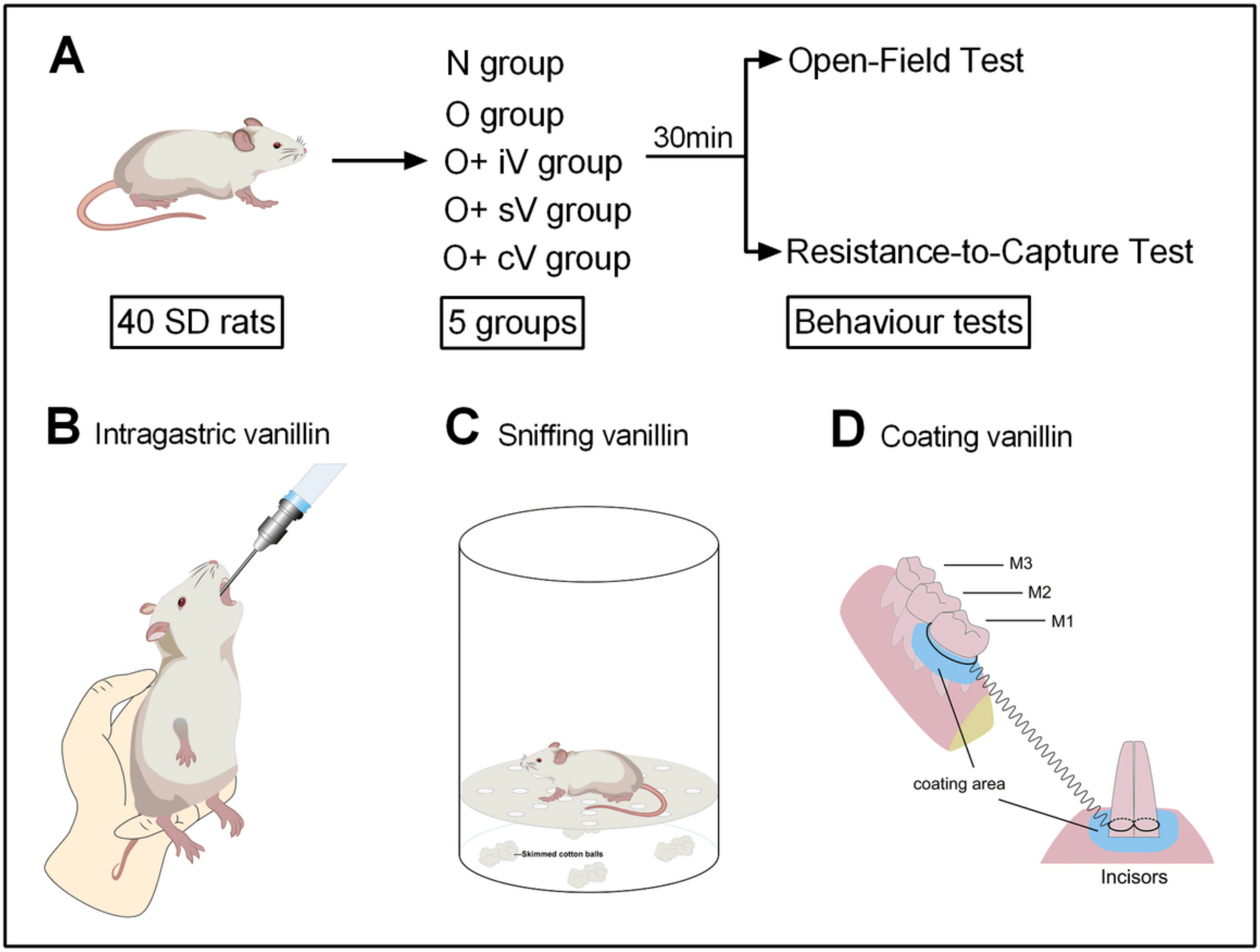

Figure 1

Experimental procedures. **(A)** Rats were divided into five groups for behavioural tests. **(B)** Vanillin solution was administered intragastrically to the rats. **(C)** Rats in a container filled with vanillin aroma to inhale aromatic scents. **(D)** Vanillin solution was coated 5 mm around the gingivalmargin of the rat maxillary incisors and first molar.

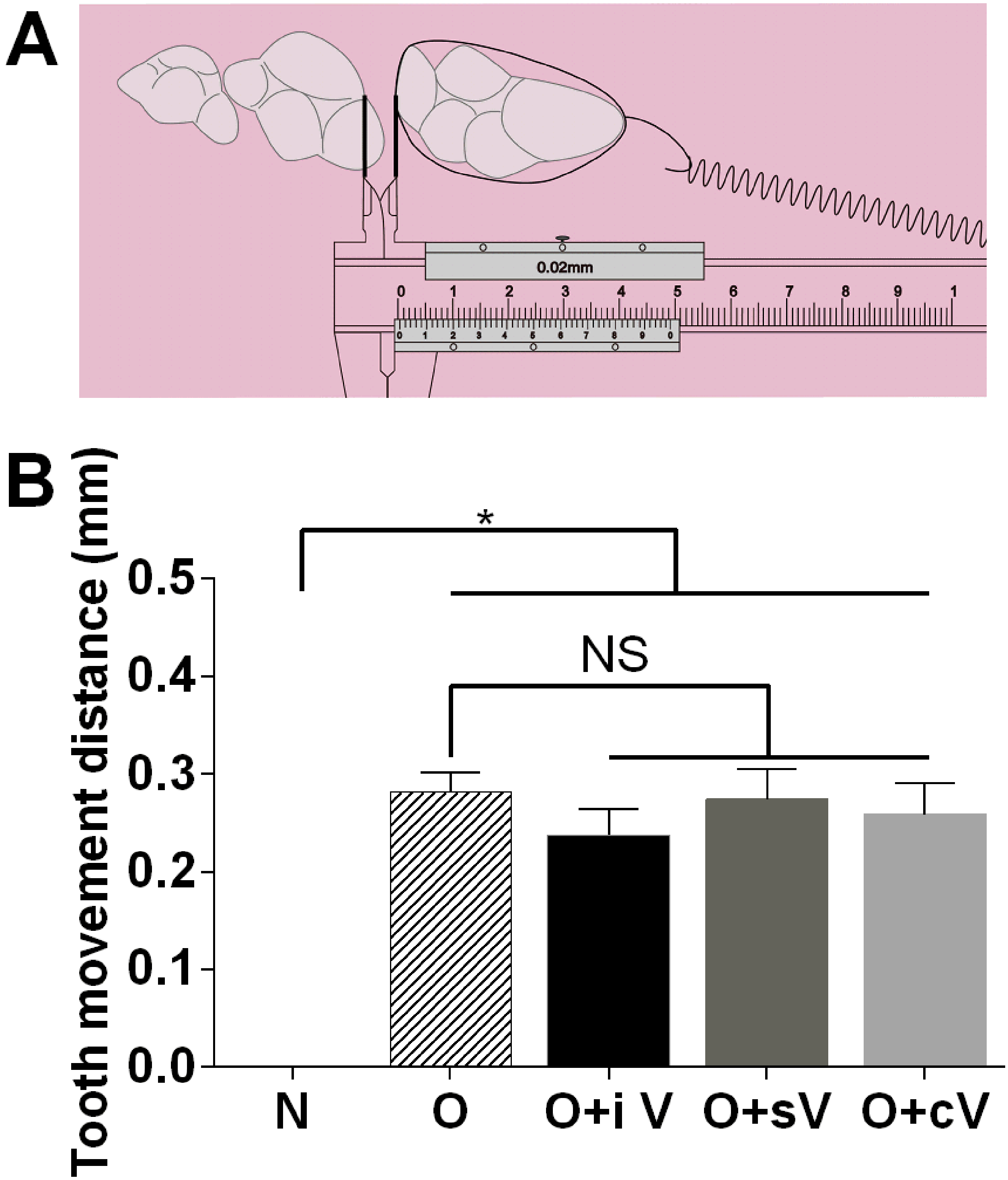

Figure 2

Tooth movement distance. (A) Distance between the maxillary first molar and maxillary second molar was measured by 0.02 accuracy vernier calipers. (B) Distances after 7 days orthodontics. Comparison with the O group. Error bars indicate SEM. *p<0.05.

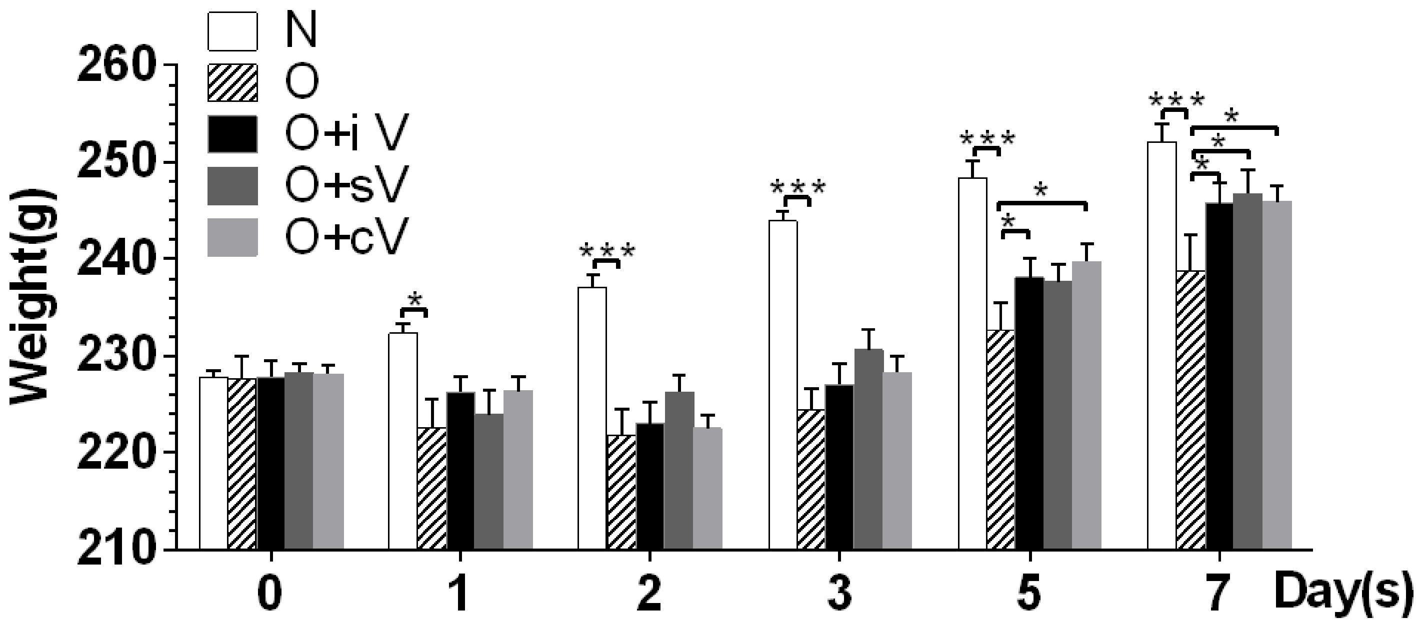

Figure 3

Weight. Weight of rats during orthodontics. Comparison with the O group; *P< 0.05, **P< 0.01, ***P< 0.001. SEM standard error of mean.

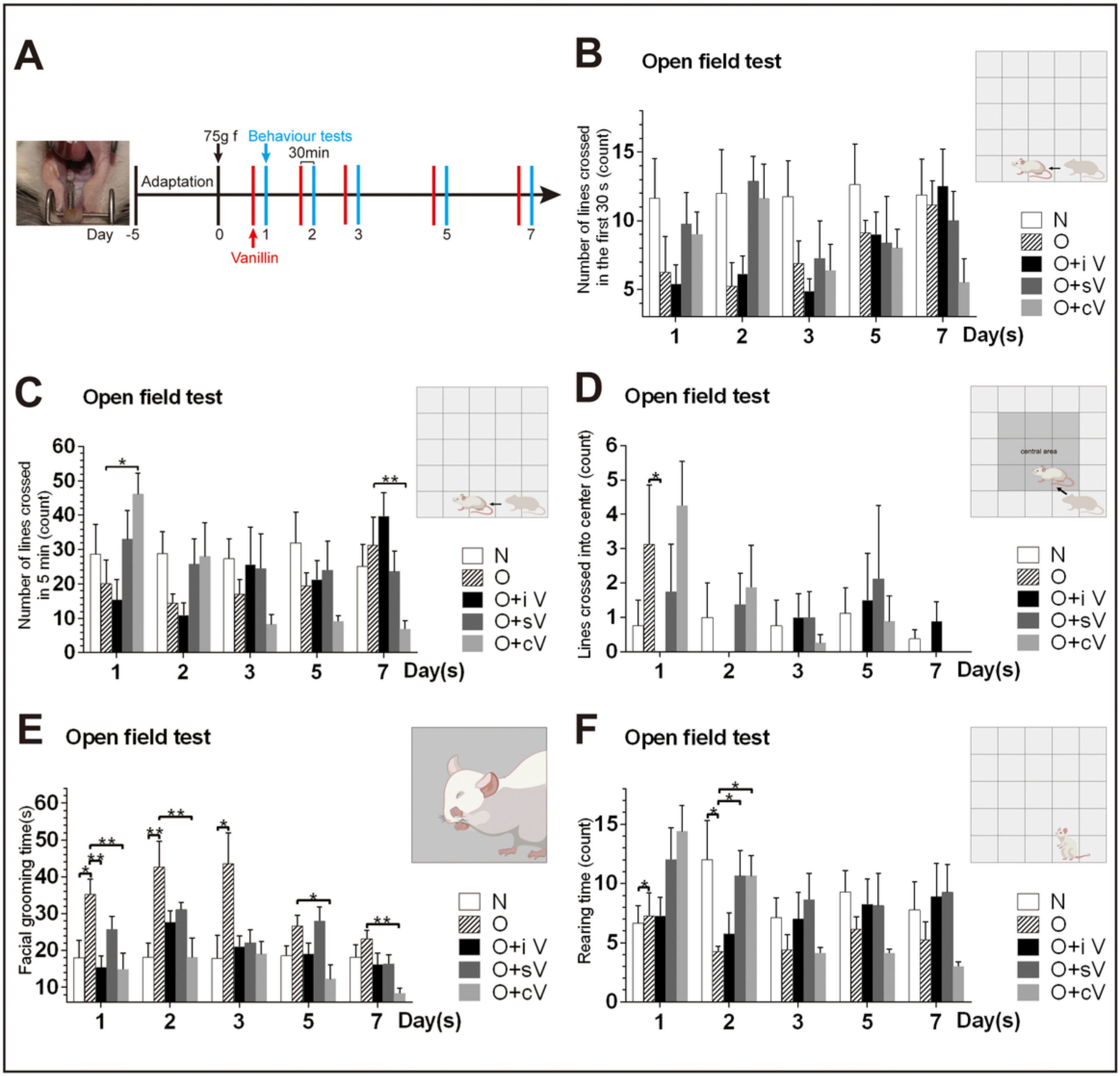

Figure 4

Open-Field Test results. Effects of administration of vanillin in rats behavioural tests. **(A)**Orthodontics was performed on the day 0. Red lines represent the time points when vanillin was administered. Blue lines represent time points when the Open-Field Tests (OFT) and Resistant-to-Capture tests were performed. Vanillin was administrated 30 minutes prior to OFT. **(B)** Lines crossed in the first 30 seconds, **(C)** lines crossed in the first 5 minutes, and **(D)**lines crossed to the center were evaluated in the open field test. **(E)**Rearing time. **(F)** Facial grooming time**.** Error bars indicate SEM. *p<0.05, **p<0.01 and ***p<0.001 vs. O group.

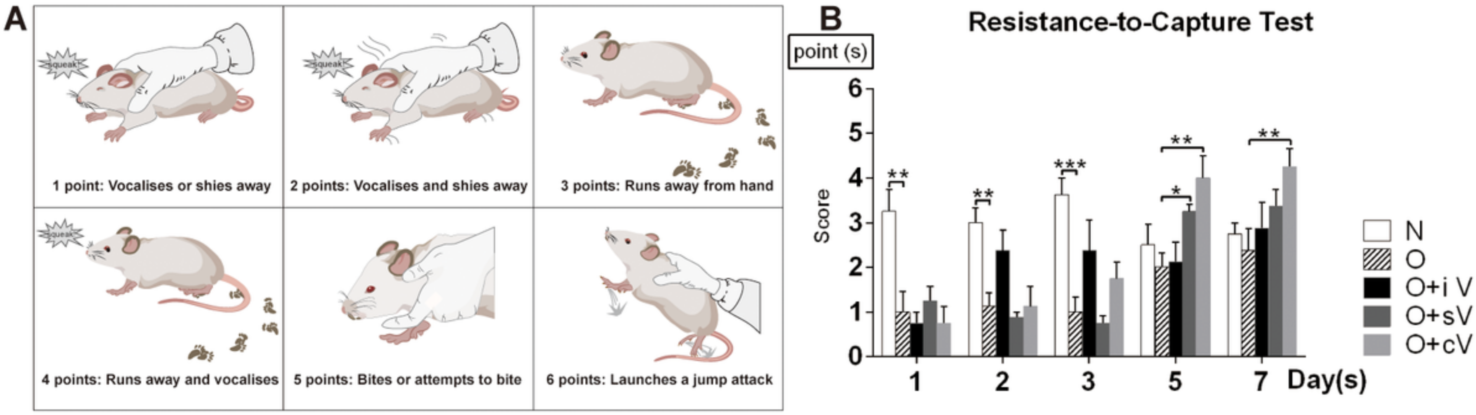

Figure 5

Resistance-to-Capture Test results. (A) The level of resistance was evaluated as follows: 0, easy to pick up; 1, vocalises or shies away from hand; 2, shies away from hand and vocalises; 3, runs away from hand; 4, runs away and vocalises; 5, bites or attempts to bite; and 6, launches a jump attack. (B) Resistance-to-Capture Test scores. Error bars indicate SEM. *p<0.05, **p<0.01 and ***p<0.001 vs. O group.