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# The effects of blocking N/OFQ receptors on orofacial pain following experimental tooth movement in rats

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*Objective:* The aim of this study was to determine the effects of nociceptin/orphanin FQ peptide receptor (N/OFQ receptor) antagonist on orofacial pain induced by experimental tooth movement in rats.

*Methods:* A total of 36 male Sprague-Dawley rats weighing 200–300 g were divided into six groups: a control group, force group, force+saline intraperitoneal group, force+saline periodontal group, force+UFP-101 ([Nphe<sup>1</sup>,Arg<sup>14</sup>,Lys<sup>15</sup>]N/OFQ-NH<sub>2</sub>; antagonist for N/OFQ receptor) intraperitoneal group, and force+UFP-101 periodontal group. Closed coil springs were ligated between the upper incisors and first molar to exert an orthodontic force (40 g) between the teeth. Injectable administration dosages were 30 µl saline or 30 µl saline containing 0.03 mg/kg UFP-101. Following the injections, orofacial pain levels were assessed through directed face grooming (mouth wiping). Statistical analyses were performed in SPSS 17.0 (Statistical Package for the Social Sciences) and *p* values less than 0.05 were considered as statistically significant.

*Results:* Orofacial pain levels were significantly higher in the force group than in the control group. Orofacial pain levels differed significantly between the force group, force+saline periodontal group and force+UFP-101 periodontal group, but were similar between the control group, force+UFP-101 intraperitoneal group and force+saline intraperitoneal group. Moreover, orofacial pain levels did not differ between the force group, force+saline intraperitoneal group and force+UFP-101 intraperitoneal group.

*Conclusions:* Periodontal, but not intraperitoneal, administration of UFP-101 could alleviate orofacial pain induced by experimental tooth movement in rats, suggesting that periodontal N/OFQ receptors participate in orofacial pain induced by experimental tooth movement.

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## Introduction

Orofacial pain induced by tooth movement, simply designated as orthodontic pain, is commonly encountered in daily orthodontic practice<sup>1,2</sup> as a result of inflammatory responses.<sup>3-5</sup> Although the exact mechanisms underlying the pain response are still poorly understood, it has been documented that many neuropeptides and pro-inflammatory mediators participate in the modulation of pain.<sup>6</sup> Investigating the mechanisms by which these molecules generate

orthodontic pain could help unravel intrinsically complex underlying mechanisms.

Nociceptin/orphanin FQ (N/OFQ), an endogenous opioid peptide, plays an important role in orofacial pain.<sup>7</sup> A large body of evidence indicates that N/OFQ produces either pro- or anti-nociceptive effects, depending on the dosage and site of administration.<sup>8,9</sup> Its anti-nociceptive effects at the spinal level prevail over its pro-nociceptive effects at the supraspinal level,<sup>8</sup> which was supported by the finding that

N/OFFQ-receptor-knockout mice displayed stronger nociceptive responses against inflammatory pain.<sup>10</sup> UFP-101, [Nphe<sup>1</sup>,Arg<sup>14</sup>,Lys<sup>15</sup>]N/OFFQ-NH<sub>2</sub>, a potent and selective antagonist of N/OFFQ receptors, could competitively inhibit various N/OFFQ actions.<sup>11,12</sup> Therefore, it is conceivable that blocking N/OFFQ receptors with UFP-101 could exacerbate pain. A previous study revealed that N/OFFQ was upregulated in the trigeminal nucleus in rats following experimental tooth movement,<sup>7</sup> suggesting a potential role of N/OFFQ in the perception of orthodontic pain. To date, however, the effects of blocking N/OFFQ receptors (through applying UFP-101) on orofacial pain induced by experimental tooth movement in rats are still largely unknown. Therefore, the aim of this study was to determine the effects of UFP-101 on orofacial pain following tooth movement.

## Methods

### *Animals*

In total, 36 male Sprague-Dawley rats (200–300 g) were used in the present study. The animals were housed in standard transparent plastic cages containing soft bedding, and had free access to food and water. The room was kept temperature-controlled at 25°C and had a 12/12h light dark cycle. Measures were taken to minimise animal numbers and distress. The methods of investigating pain in animals adhered to the guidelines of The International Association for the Study of Pain.<sup>13</sup>

Fixed Ni-Ti alloy closed-coil springs were ligated between the left maxillary first molar and upper incisors, which delivered 40 g force (measured by a force meter) to generate mesial movement of the maxillary molars.

### *Drug administration*

Following general anaesthesia with 4% chloral hydrate (peritoneal injection; 3 mg/kg), force loading and subsequent drug administration was performed. The rats were randomly divided into six groups: a control group, a force group, a force+saline intraperitoneal group, a force+UFP-101 intraperitoneal group, a force+saline periodontal group, and a force+UFP-101 periodontal group. The spring appliances were mounted in the mouths of the control group but inactivated so that no forces were applied. The spring

appliances were mounted and activated in the force groups so that a force of 40 g was delivered. Each rat in the force+saline intraperitoneal group received an intraperitoneal injection of 30 µl saline one day after force loading. Each rat in the force+UFP-101 intraperitoneal group received an intraperitoneal injection of 30 µl UFP-101 solution (0.03 mg/kg) after the force loading. Each rat in the force+saline periodontal group received a periodontal injection of 30 µl saline one day after the force loading. Each rat in the force+UFP-101 periodontal group received a periodontal injection of 30 µl UFP-101 solution (0.03 mg/kg) after the force loading. For each rat, the periodontal injections were performed at the following six sites with 5 µl administered at each site (totalling 30 µl): buccal, palatal and distal sites of the incisors; buccal, palatal and mesial sites of the first molars.

### *Evaluation of pain*

Orthodontic pain levels were assessed through directed face-grooming (mouth wiping) according to the protocols established in a previous study.<sup>5</sup> Four hours after drug administration, the rats were placed into transparent plastic cages (30 cm × 30 cm × 30 cm) with 45-dB of background noise. Face-grooming activities were video-taped for three sessions (10 minutes for each). The videos were analysed by two authors who were blinded to the animal groups. The mouth wiping time was recorded and considered as a surrogate for orofacial pain generated following experimental tooth movement.

### *Statistics*

A Rank Sum Test was used for statistical analyses in SPSS 17.0 (Statistical Package for the Social Sciences). A *p* value less than 0.05 was considered as statistically significant.

## Results

As presented in Figure 1 and Table I, the results showed that pain levels were significantly higher in the force group (median: 62.3; interquartile range: 58.5) than in the control group (median: 11.3; interquartile range: 25.7), indicating that orthodontic force could induce orofacial pain in rats.

As displayed in Figure 2 and Table I, it was found that pain levels were similar between the force group

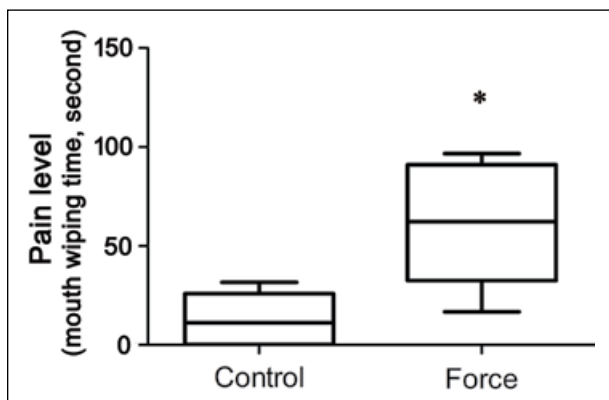


Figure 1. Orofacial pain levels (mouth wiping time, seconds) in the force group and the control group following experimental tooth movement. The plus and minus error bars stand for maximal and minimal values, respectively. The box stands for interquartile range and the horizontal line through the box stands for the median value.

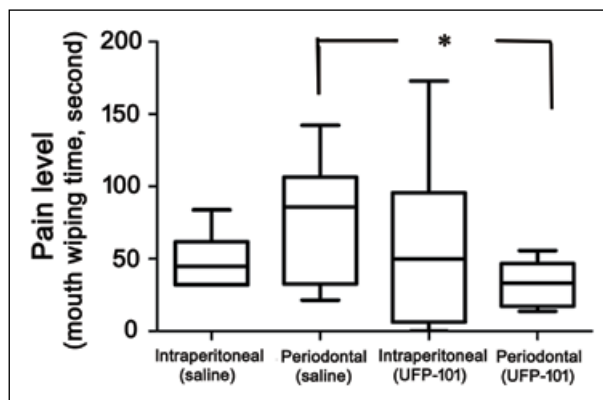


Figure 2. Orofacial pain levels (mouth wiping time, seconds) in saline periodontal group, UFP-101 periodontal group, saline intraperitoneal group and UFP-101 intraperitoneal group. The plus and minus error bars stand for maximal and minimal values, respectively. The box stands for interquartile range and the horizontal line through the box stands for the median value.

Table I. Mouth wiping (pain level) following experimental tooth movement in rats.

Group	Control	Force	Saline i.p. group	Saline p. group	UFP-101 i.p. group	UFP-101 p. group
Median	11.2900	62.2850	44.6800	85.7950	49.8500	33.1500
Interquartile range	25.685	58.505	29.655	73.9225	89.3775	29.52

i.p.: intraperitoneal  
p.: periodontal

(median: 62.3; interquartile range: 58.5) and both of the intraperitoneal groups (saline – median: 44.7; interquartile range: 29.7 – and UFP-101 – median: 49.9; interquartile range: 89.4), while pain levels differed between the force group (median: 62.3; interquartile range: 58.5) and both of the periodontal groups (saline – median: 85.8; interquartile range: 73.9 – and UFP-101 – median: 33.2; interquartile range: 29.5). In addition, pain levels did not differ between the force+saline intraperitoneal group (median: 44.7; interquartile range: 29.7) and the force+UFP-101 intraperitoneal group (median: 49.9; interquartile range: 89.4). In contrast, pain levels were significantly higher in the force+UFP-101 periodontal group (median: 33.2; interquartile range: 29.5) than in the force+saline periodontal group (median: 85.8; interquartile range: 73.9).

### Discussion

Orofacial pain is commonly encountered in patients during orthodontic treatment particularly after the placement and adjustment of appliances. It has been well documented that orthodontic pain is associated

with pressure, ischaemia, inflammation and oedema.<sup>14</sup> It has been further shown that released molecules<sup>15</sup> (substance P and calcitonin-related gene peptide (CGRP)) are closely associated with orthodontic tooth movement. Clinically, the initial orthodontic pain and discomfort peaks during the first three days following appliance activation. Pain begins to fade after one week.<sup>16</sup> Therefore, the present research chose to compare pain levels between the different animal groups on the first day after the application of force. Experiment tooth movement produced an obvious animal behaviour characterised by directed face-grooming.<sup>17</sup> Similar directed grooming responses have been reported as a result of noxious stimulation applied to rats.<sup>18-20</sup> A previous study found that both systemic and local administrations of morphine could relieve orthodontic pain in rats.<sup>5</sup> Moreover, local administration exerts a better analgesic effect than systemic administration following experimental animal tooth movement, which supports the clinical use of peripheral anti-nociceptive medicine in the control of orthodontic pain.<sup>5</sup>

Similar to other classical opioid ligands, N/OFQ is an endogenous opioid peptide whose receptor is

located in the central but also the peripheral nervous system. The peptide plays an important role in the modulation of pain and gastrointestinal function.<sup>9</sup> The effects are, however, controversial. The effects of N/OAQ administration could vary because of dosage and the site of administration. Intrathecal (IT) blockade of N/OAQ receptors was not shown to alleviate acute pain in previous rat models.<sup>18</sup> However, IT injection of N/OAQ receptor antagonists was able to reverse the nociceptive response of electroacupuncture in inflammatory rat models.<sup>21</sup> In addition, the use of a peripheral N/OAQ receptor agonist could produce an anti-nociceptive effect in capsaicin-induced inflammatory pain models. A previous study revealed that N/OAQ was upregulated in the trigeminal nucleus in rats following tooth movement.<sup>7</sup> However, the exact role of N/OAQ in the regulation of orthodontic pain following experimental tooth movement is unknown. In the present study, UFP-101 was used to block N/OAQ, both locally and systemically. In Carvalho's research,<sup>22</sup> the 0.03 mg/kg and 0.3 mg/kg administration of UFP-101 significantly reduced mortality after surgery involving cecal ligation and puncture (CLP), but dosages over 0.1 mg/kg increased the mortality of rats. Therefore, a dosage of 0.03 mg/kg for UFP-101 was used in the present study.

The present study found that the periodontal administration of UFP-101 relieved orthodontic pain in rats, indicating that peripheral N/OAQ produces pro-nociceptive effects. The anti-nociceptive effects of N/OAQ at the spinal level prevailed over its pro-nociceptive effects at the supraspinal level.<sup>8</sup> Therefore, it is conceivable that systemic blocking N/OAQ receptors with UFP-101 produced pro-nociceptive effects on orthodontic pain. However, it was found that the systemic administration of UFP-101 had no effect on orthodontic pain. This could be attributed to the anti-nociceptive effects of UFP-101 at periodontal sites, which may offset the net pro-nociceptive effects of UFP-101 produced at the spinal and supraspinal levels. This would hold provided that UFP-101 reached periodontal, spinal and supraspinal sites following systemic administration. The possibility suggests that N/OAQ may produce local effects in addition to both spinal and supraspinal effects.

In conclusion, it is suggested that N/OAQ receptors mediate pro-nociceptive effects at periodontal sites on orthodontic pain and that periodontal, rather than

systemic, blocking of N/OAQ receptors with UFP-101 could relieve orthodontic pain in rats.

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