

## Emotional Stress- and Pain-Related Behaviors Evoked by Experimental Tooth Movement

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### ABSTRACT

**Objective:** To investigate by behavioral methods the relationship between emotional stress and pain during experimental tooth movement in rats.

**Materials and Methods:** Sixteen male Sprague-Dawley rats (210 to 250 g) were divided into two groups. The experimental group was treated with an active Ti-Ni appliance, and the control group received a passive appliance. A force of 20 gf was delivered by the active appliance between the maxillary first and second molars for 3 days. During this period the rat's behavior was evaluated eight times by means of open-field test and resistance-to-capture test. The specific parameters of animal activity were facial grooming, rearing, and locomotor activity, movement into the center of the open field, and response to capture.

**Results:** Parameters related to stress and pain were higher in the group carrying active appliance, compared to the group with a passive appliance. Statistically significant differences in stress-related behavior between control and experimental groups were found 8 hours after placing the appliance and were most evident on the second day. Pain-related behavior was significantly greater in the experimental group than in the control group at 24 hours.

**Conclusions:** The increase in emotional stress evoked by orthodontic tooth movement may precede the appearance of periodontal pain.

**KEY WORDS:** Stress; Anxiety; Pain; Animal behavior; Orthodontics; Rat

### INTRODUCTION

The discomfort, pain and stress frequently associated with orthodontic treatment are often underesti-

mated by orthodontists and feared by patients. These side effects can produce a considerable amount of distress in the daily life of patients, such as difficulties in chewing and biting.<sup>1</sup> Furthermore, 10% of patients choose to terminate their therapy,<sup>2</sup> and potential orthodontic patients avoid treatment due to the fear of feeling pain.<sup>3</sup>

There are few studies about the emotional stress and anxiety that patients endure during orthodontic treatment. Several studies compare the emotional status of patients before and after orthodontic treatment, and show a positive outcome.<sup>4</sup> However, it is of interest to assess the stress and anxiety and their relationship with pain during orthodontic treatment. Therefore, the purpose of this study was to investigate by behavioral testing the emotional stress in relation to pain evoked by experimental tooth movement in rats.

### MATERIALS AND METHODS

All possible measures were taken to minimize animal suffering and the number of animals used in this study. The experimental procedures described here followed the Guidelines for Animal Research and had the prior approval from the Animal Welfare Committee of Nagasaki University.

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## Animals

Sixteen male Sprague-Dawley rats (210–350 g) were housed in pairs in plastic cages in a colony room following a 12-hour light:dark cycle with an ambient temperature maintained at 21°–23°C. Food and water were available ad libitum. After arrival, the rats were allowed to habituate to the colony room for 3 to 5 days before the experiments began.

## Experimental Design

The animals were divided into two groups: experimental and control ( $n = 8$ , each). Active and passive intraoral springs were bonded to the upper first and second molars of experimental and control animal groups, respectively. The rats then went through behavioral tests eight times in the following 49 hours. To assess whole body effects the animals were weighed before treatment and on regular intervals during the experimental period.

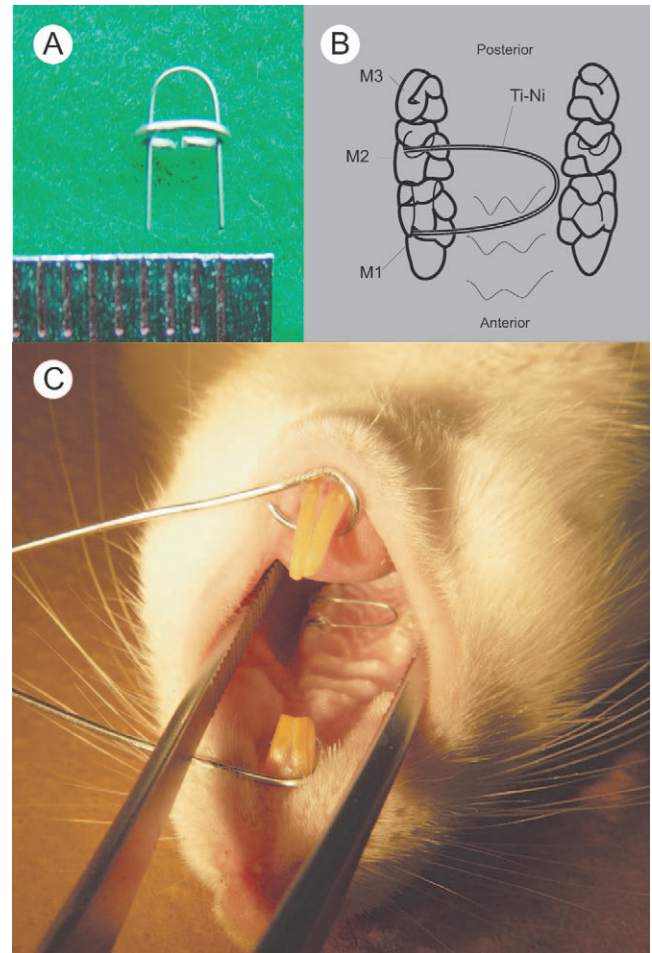
## Experimental Tooth Movement Model

The rats were anesthetized intraperitoneally with ketamine hydrochloride in combination with xylazine hydrochloride with a dosage of 87 mg/kg for ketamine and 13 mg/kg for xylazine (Ketalar 50, Sankyo Co, Ltd, Tokyo, Japan; and Celactal 2% injections, Bayer-Japan Co, Ltd, Tokyo, Japan).

Buccopalatal grooves were cut with a steel bur (no. 0.5, Maillefer, Swiss) on the right maxillary first (M1) and second (M2) molars' occlusal surfaces.<sup>5</sup> The cutting was performed under water and air spray cooling. The distance between both grooves was 3 mm, and the depth of each groove was just enough to seat the spring wire (0.3 mm). The site was dried, etched with 65% phosphoric acid for 20 seconds, rinsed with water, and dried again.

The wire used for both active and passive appliances was a work-hardened titanium-nickel alloy (Ti-Ni) measuring 0.228 mm in diameter and 14 mm in length (TOMY, Tokyo, Japan) (Figure 1). The wire to be used as a passive appliance (control group) was bent at its center (by loop forming pliers) and heat-treated so that the ends were at a 3-mm distance to one another. The wire used as an active spring (experimental group) was initially straight (not bent). An initial force of 20 gf was delivered by the active appliance.

In preparation for bonding the spring, the tips were brought together and maintained at a distance of 3 mm by a circular frame. The frame was removed to activate the spring after the spring was bonded. Finally, the springs were seated into the occlusal grooves and bonded in place with cyanoacrylate glue. Afterwards, the rats were allowed to recover from anesthesia and returned to their cages in the colony room.



**Figure 1.** Experimental appliance. (A) Spring held in activated position in preparation for bonding (scale in mm). (B) Detailed schematic of the occlusal aspect of the rat's molars with the experimental appliance. (C) Photograph of the appliance bonded in place.

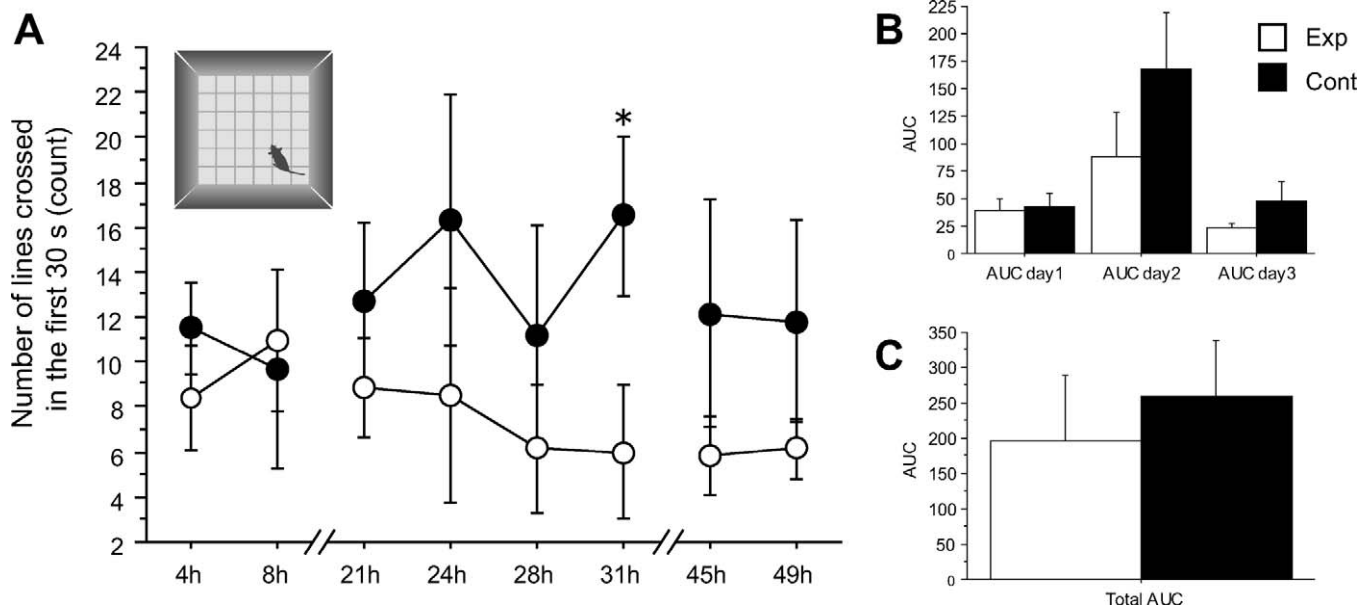
## Behavioral Testing

All behavioral testing occurred during the light phase of the light:dark cycle. The tests were performed 4, 8, 21, 24, 28, 31, 45, and 49 hours after placing the appliances. The rats were brought one by one to the test room (next to the colony room) for behavioral testing and returned to the colony room immediately afterwards. The test room was quiet and temperature controlled (22°C).

In all tests, the animal's response was recorded on videotape and later analyzed by an observer blinded to the animals' group assignment. The video camera was positioned vertically 2 m above the test field.

## Open-Field Test

The rat was placed at one corner of a 70 × 70 cm open field surrounded by 30-cm high cardboard walls. The floor was made of Plexiglas divided by white adhesive tape into 36 squares of identical size. The an-



**Figure 2.** Lines crossed in the first 30 seconds of the open-field test. A: time course; B and C: area under the curve analysis from A. Rats in the experimental group showed lower activity than those in the control group. AUC indicates area under the curve; Exp, experimental group; Cont, control group. \*  $P < .05$  in the Mann-Whitney  $U$ -test. Error bars represent SEM.

imals' behavior in the open field was recorded for 5 minutes.<sup>6</sup> The following parameters were analyzed: (1) number of lines crossed in the first 30 seconds, (2) total number of lines crossed in 5 minutes, (3) number of lines crossed to the center of the open field, (4) rearing time, and (5) facial grooming time. A line was considered crossed when all four paws crossed it. Rearing and facial grooming time was measured with a stopwatch and consisted of the cumulative time of rearing and facial grooming episodes, respectively.

### Resistance-to-Capture Test

After the open-field test, we performed the resistance-to-capture test. The test consisted of measuring the animals' resistance to being picked up by the examiner. The level of resistance was evaluated as follows: 0, easy to pick up; 1, vocalizes or shies away from hand; 2, shies away from hand and vocalizes; 3, runs away from hand; 4, runs away and vocalizes; 5, bites or attempts to bite; and 6, launches a jump attack.<sup>7</sup>

### Data Analysis

Data from experimental and control groups were compared by the Mann-Whitney  $U$ -test. The data from each time point were grouped as the area under the curve (AUC) for the first, second, and third days, and for the whole experimental period. The AUCs for day 1 and day 3 comprise two data points, or 4 hours, and for day 2, 4 data points, or 10 hours. The night hours

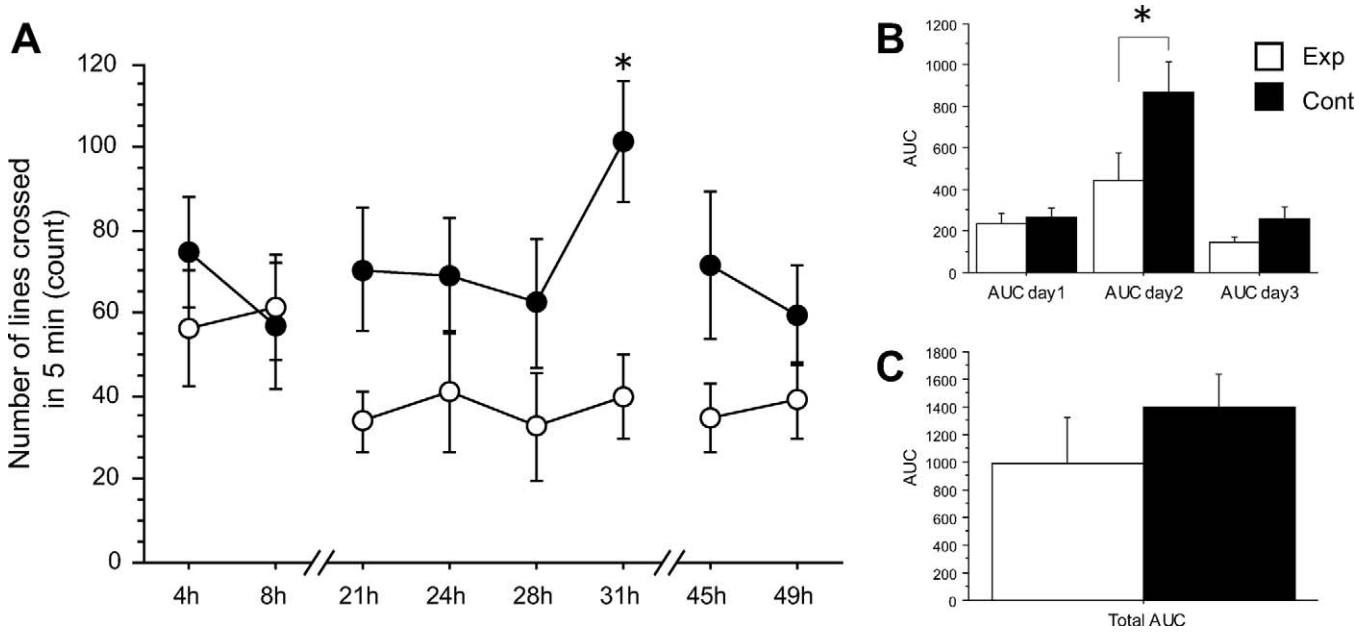
were omitted from the AUC analysis. The significance level was set at  $P < .05$ . Data are displayed as mean values with the respective  $\pm$  standard error of the mean (SEM).

## RESULTS

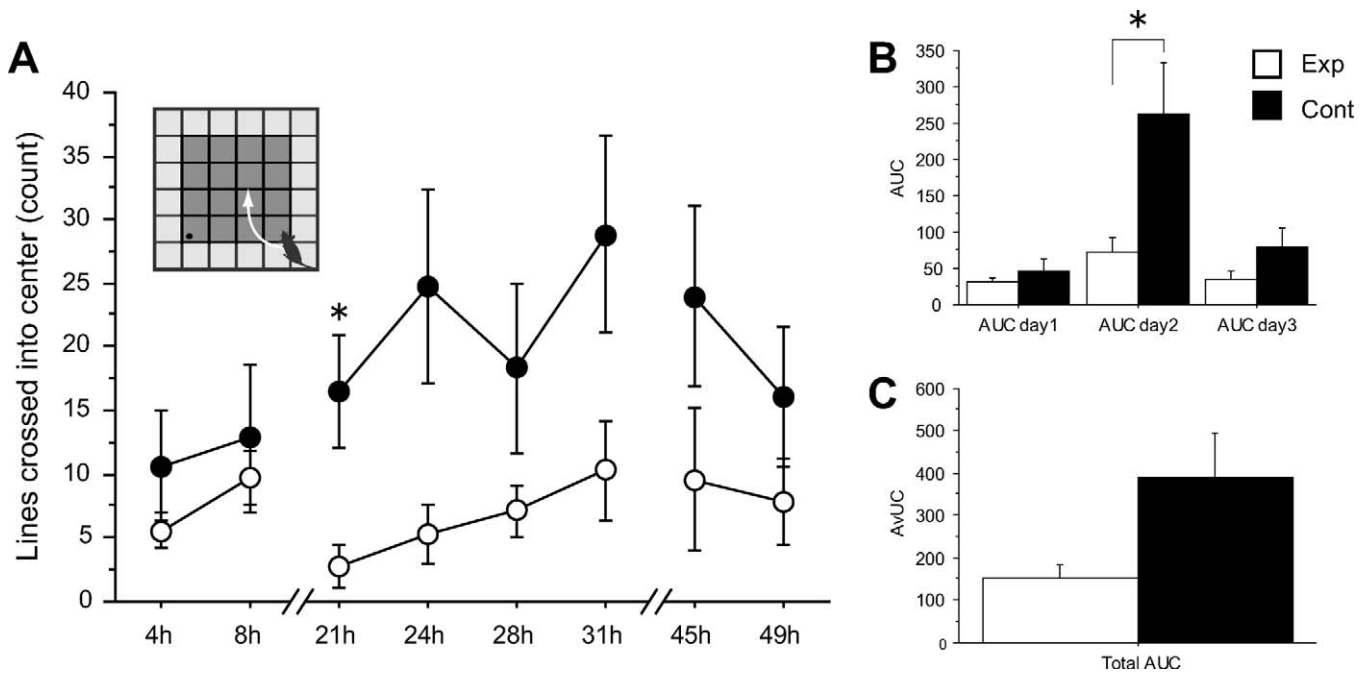
Body weight did not differ significantly between experimental and control animals. In both groups, the mean value of body weight decreased slightly from  $289.7 \pm 11.9$  g ( $289.5 \pm 17.0$  g in experimental and  $290.0 \pm 18.0$  g in control) before surgery to  $279.2 \pm 12.7$  g ( $276.0 \pm 17.6$  g in experimental and  $282.0 \pm 19.3$  g in control) 12 hours after surgery. After that the body weight increased continuously and steadily, reaching  $287.3 \pm 12.1$  g ( $282.8 \pm 17.3$  g in experimental and  $291.2 \pm 18.0$  g in control).

### Open-Field Test

The activity during the first 30 seconds (Figure 2) of the 5-minute open-field test showed a tendency for the experimental group to be less explorative during this initial phase, especially on day 2, with a statistical significance at  $t = 31$  h. Total ambulation during the 5 minutes of testing period was lower in the experimental group compared to the control group on day 2 (Figure 3). A statistically significant difference was observed at  $t = 31$  h and on day 2 (AUC from 21 h to 31 h). Likewise, analysis of the number of lines crossed into the central area showed that rats in the control group went into the central area more often



**Figure 3.** Lines crossed in the 5 minutes of the open-field test. A: time course; B and C: area under the curve analysis from A. Lower values were more often associated with the experimental group. AUC indicates area under the curve; Exp, experimental group; Cont, control group. \*  $P < .05$  in the Mann-Whitney  $U$ -test. Error bars represent SEM.

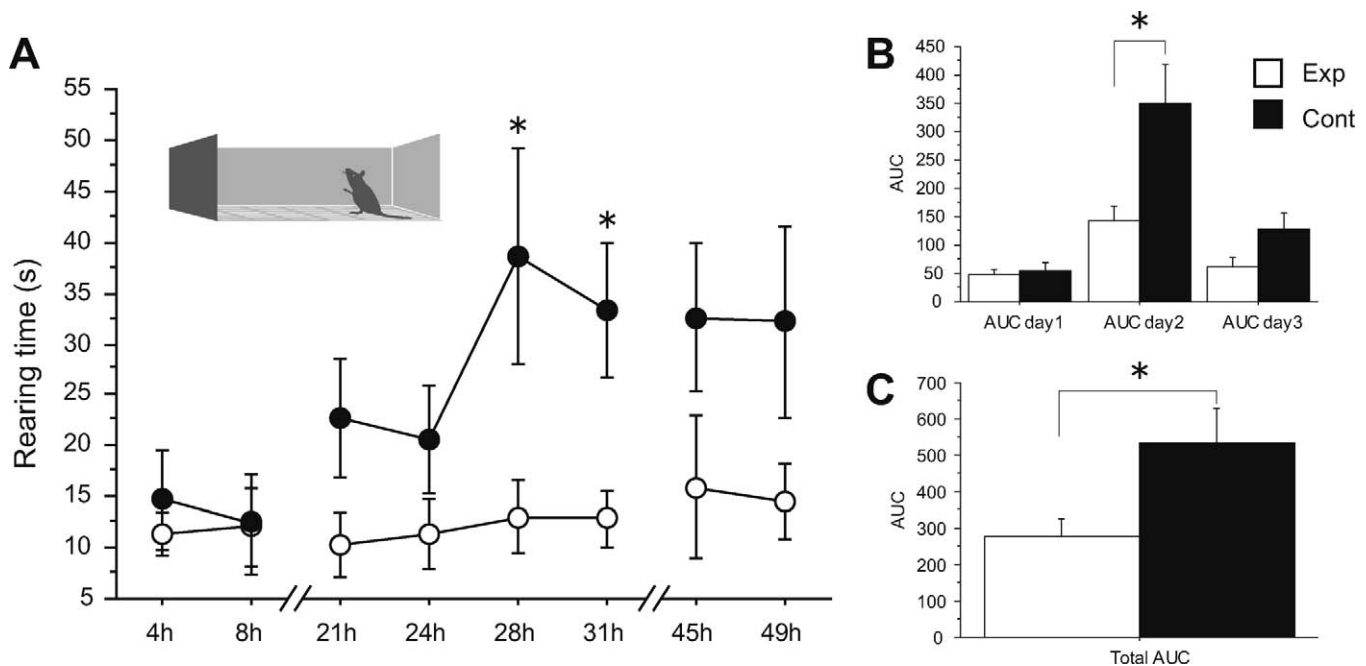


**Figure 4.** Lines crossed into the center of the open field. A: time course; B and C: area under the curve analysis from A. Lower values were more often associated with the experimental group. AUC indicates area under the curve; Exp, experimental group; Cont, control group. \*  $P < .05$  in the Mann-Whitney  $U$ -test. Error bars represent SEM.

than those in the experimental group (Figure 4). This difference was statistically significant at  $t = 21$  h as well as for day 2 (AUC). Rearing time in the open field was less in the experimental group (Figure 5). Compared with the control group, there were statistically significant differences at  $t = 28$  h and  $t = 31$  h, and

in the AUC analysis at day 2 and for the total duration of the experiment.

Facial grooming activity during the open-field test showed a tendency to increase on the second and third days for both the experimental and control groups (Figure 6). In the experimental group facial grooming



**Figure 5.** Rearing time in the open-field test. A: time course; B and C: area under the curve analysis from A. Rearing activity in the experimental group was markedly reduced compared to the experimental group. AUC indicates area under the curve; Exp, experimental group; Cont, control group. \*  $P < .05$  in the Mann-Whitney  $U$ -test. Error bars represent SEM.

time was greater on the second day than the control group. A statistically significant difference was observed between the two groups at  $t = 24$  h ( $P < .05$ ).

### Resistance-to-Capture Test

The resistance-to-capture test scores decreased over time for both experimental and control groups (Figure 7). The difference between groups was found to be statistically significant at  $t = 8$  h. Although the mean score values were considerably lower in the control group throughout, there were no statistically significant differences in the AUC analysis for any particular day nor for the total experimental period.

## DISCUSSION

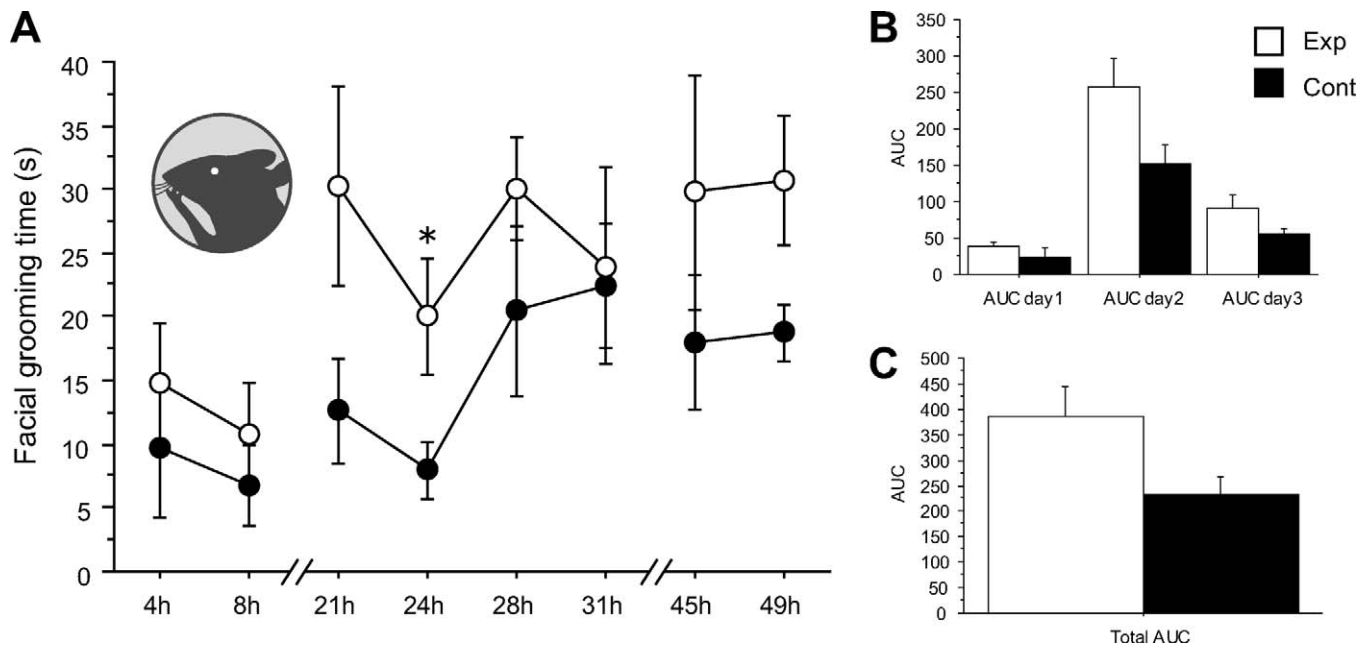
In order to simulate an actual orthodontic treatment, we attached springs to the rat maxillary first and second molars. Then, for the following 49 hours the stress response was estimated by the rats' behavior in the open-field and resistance-to-capture tests. The open-field test has been used for over 70 years in the assessment of genetic and environmental factors as well as the effects of drugs on animal emotionality.<sup>8</sup> The resistance-to-capture test is a common test used in rats for the assessment of emotional stress.<sup>9</sup>

### Open-Field Test

In the open-field test on the second day, parameters related to ambulatory activity were markedly reduced

in rats with an active appliance (experimental group) compared to those with a passive appliance (control group). This result indicated a higher level of anxiety in experimental rats at this time point. In the first 30 seconds of the open-field test, the animals are challenged with the sudden change from a familiar environment to a novel one (from the cage in the colony room to the open field in the test room), and anxiety-related reduction in the number of lines crossed is greater during this period.<sup>10</sup> General ambulatory activity is believed to be reduced in animals subjected to stress and anxiety, but analysis of exploratory behavior (number of lines crossed towards the center of the open field) may help to further distinguish the emotional component within the locomotor activity.<sup>11</sup> In this study, lines crossed into the center and rearing activity showed a significant difference between experimental and control groups on the second day (AUC), which emphasizes the higher level of stress and anxiety in rats from the experimental group 1 day after appliance placement.

It is well known that active orthodontic appliances cause pain in human patients. Likewise, experimental tooth movement in rats has been shown to produce *c-fos* expression (a marker for neuronal activity) in the trigeminal subnucleus caudalis and parabrachial nucleus, regions involved in the transmission of nociceptive information.<sup>12</sup> In this study 24 hours after placement of the appliances, there was significantly greater facial grooming activity in the experimental group com-



**Figure 6.** Facial grooming time measured during the 5 minutes of the open-field test. A: time course; B and C: area under the curve analysis from A. Facial grooming activity was greater in the experimental group compared with the control group. AUC indicates area under the curve; Exp, experimental group; Cont, control group. \*  $P < .05$  in the Mann-Whitney  $U$ -test. Error bars represent SEM.

pared with the control group. Excessive facial grooming in rats is a characteristic behavior indicative of orofacial pain.<sup>13</sup> Therefore, the significantly greater facial grooming activity in the experimental rats may have coincided with the peak of pain sensation evoked by the mechanical force exerted from the activated spring on the periodontal ligaments.<sup>1,12,14</sup>

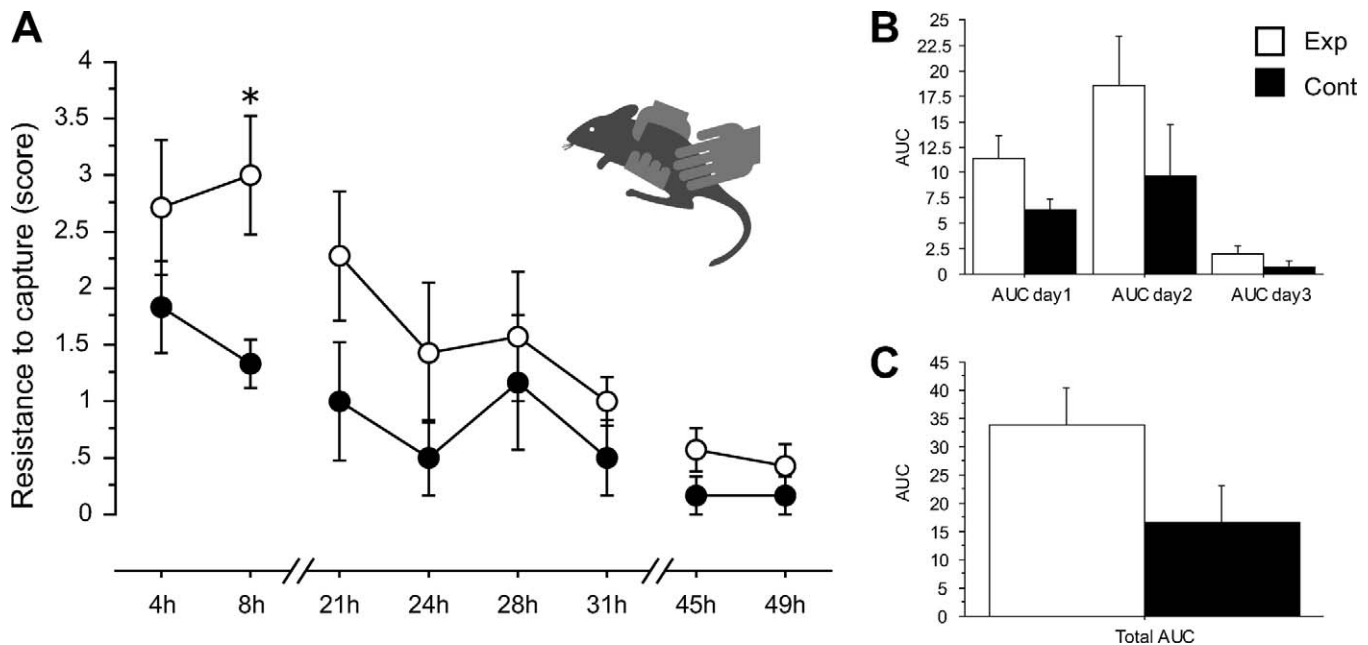
### Resistance-to-Capture Test

On the resistance-to-capture test, stressed rats are more likely to respond aggressively to being picked up by the examiner,<sup>7</sup> and thus receive a higher scoring. Extremely stressed rats generally have a mean resistance to capture of 5, whereas control rats usually have a mean resistance to capture around 1.<sup>9</sup> Eight hours after placement of the appliances the experimental group received significantly higher scores, compared to the control group. This result indicates that rats with an active appliance were experiencing a higher level of stress in the resistance-to-capture test. This result is in agreement with a previous study in which rats with occlusal disharmonies showed increased levels of stress hormones peaking at about 6.5 to 8.5 hours.<sup>15</sup> In our study, however, rats in both groups showed scores above 1 on the first day, and the scores gradually decreased for both groups being close to zero on the third day. This tendency suggests that the resistance-to-capture test was very sensitive for detecting a difference in the stress response between groups as early as 8 hours after placement of

the appliances, but also that the rats gradually habituated after repetitive testing.

### Mechanisms of the Stress Response

The differences in stress-related behavior between control and experimental rats have been due to at least two reasons. First, it is likely that changes in occlusal contacts as well as the modified perception of occlusal forces through the compressed periodontal ligaments may have caused a greater discomfort in rats in the experimental group. It has been shown that in rats experimental tooth movement affects periodontal sensation,<sup>16</sup> and that occlusal disharmony is known to increase the release of stress hormones causing chronic emotional stress in rats and monkeys.<sup>15,17</sup> In patients, the lack of stable occlusal relationships is a considerable source of discomfort during the initial stages of the orthodontic treatment, and this discomfort may translate into increased levels of stress even before the appearance of pain.<sup>18</sup> Second, the effect of the force being transmitted from the spring wire to the periodontal ligament of the first and second molars may have caused considerable pain. Several studies in both humans<sup>1,14,19,20</sup> and experimental animals<sup>12</sup> agree that the application of moderate to heavy mechanical force to the teeth causes pain sensation with a peak at 24 hours and lasting for 2 to 3 days. It has been shown that patients with facial pain have high daytime levels of cortisol,<sup>21</sup> which is one of the effects of the stress response.



**Figure 7.** Resistance-to-capture test. A: time course; B and C: area under the curve analysis from A. At the end of the 5-minute open-field test, each animal's resistance to being captured was assessed. AUC indicates area under the curve; Exp, experimental group; Cont, control group. \*  $P < .05$  in the Mann-Whitney  $U$ -test. Error bars represent SEM.

## CONCLUSIONS

- Orthodontic tooth movement may elicit emotional stress ahead of pain sensation.
- Emotional stress during orthodontic treatment may be caused by several factors and possibly influence the experience of pain.

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