Annals of Anatomy 195 (2013) 539–553

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.de/aanat

RESEARCH ARTICLE

# Differentiated analysis of orthodontic tooth movement in rats with an improved rat model and three-dimensional imaging

Christian Kirschneck[a,*], Peter Proff[a], Jochen Fanghaenel[a], Michael Behr[b], Ulrich Wahlmann[c], Piero Roemer[a]

[a] Department of Orthodontics, University Medical Centre Regensburg, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany
[b] Department of Prosthodontics, University Medical Centre Regensburg, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany
[c] Department of Oral and Maxillofacial Surgery, University Medical Centre Regensburg, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany

ARTICLE INFO

*Article history:*
Received 5 August 2013
Received in revised form 26 August 2013
Accepted 28 August 2013

*Keywords:*
Wistar rats
Tooth movement
Orthodontics
Orthodontic model
Cone beam computed tomography
Cephalometry
Animal models

SUMMARY

Rat models currently available for analysis of orthodontic tooth movement often lack differentiated, reliable and precise measurement systems allowing researchers to separately investigate the individual contribution of tooth tipping, body translation and root torque to overall displacement. Many previously proposed models have serious limitations such as the rather inaccurate analysis of the effects of orthodontic forces on rat incisors. We therefore developed a differentiated measurement system that was used within a rat model with the aim of overcoming the limitations of previous studies.

The first left upper molar and the upper incisors of 24 male Wistar rats were subjected to a constant orthodontic force of 0.25 N by means of a NiTi closed coil spring for up to four weeks. The extent of the various types of tooth movement was measured optometrically with a CCD microscope camera and cephalometrically by means of cone beam computed tomography (CBCT).

Both types of measurement proved to be reliable for consecutive measurements and the significant tooth movement induced had no harmful effects on the animals. Movement kinetics corresponded to known physiological processes and tipping and body movement equally contributed to the tooth displacement. The upper incisors of the rats were significantly deformed and their natural eruption was effectively halted.

The results showed that our proposed measurement systems used within a rat model resolved most of the inadequacies of previous studies. They are reliable, precise and physiological tools for the differentiated analysis of orthodontic tooth movement while simultaneously preserving animal welfare.



## 1. Introduction

Great advances in orthodontic treatment have been made in the 100 years since the teachings of Angle. However, these advances were often discovered by means of trial and error in the process of treating patients in clinical practice so that only little evidence-based data are available. Current orthodontic research focuses on physiological and biological principles of orthodontic tooth movement that are not yet fully understood (Brooks et al., 2009; Lacey et al., 1998; Tomizuka et al., 2007). The main purpose of this research is to find ways to influence these molecular mechanisms to improve orthodontic treatment (Fujita et al., 2008; Kanzaki et al., 2006; Ralte et al., 2011). Because of their highly experimental nature, such manipulations pose a great risk to patients. Therefore, the safe usage of these manipulations needs to be thoroughly tested beforehand because results from *in vitro* experiments (Roemer et al., 2012) are often not applicable to humans. Current orthodontic research therefore relies on *in vivo* experiments with animal models to obtain evidence-based results for current treatment practices (Okamoto et al., 2009; Boas Nogueira et al., 2012) and to develop new treatment methods (Hashimoto et al., 2001; Igarashi et al., 1994; Ortega et al., 2012).

Rats (*Rattus norvegicus*) have been proven to be the most suitable animals for orthodontic research, both for studies on tooth movement (Ren et al., 2004) and cranial development (Fanghänel and Miehe, 1994; Bienengräber et al., 1999; Weingärtner et al., 2007). Rats are also most suitable for studies on the anatomical structures on which tooth movement and cranial development are based (Arambawatta et al., 2005; Inoue et al., 2012, 2013; Kakei et al., 2007; Yamamoto et al., 2000, 2001). The suitability of rats is confirmed by their widespread use: a MEDLINE research project conducted by Ren et al. (2004) using the keywords “orthodontics” AND “tooth movement OR tooth displacement” AND “rat OR rats” yielded a total of 175 studies, although only studies from 1981

* Corresponding author. Tel.: +49 941 944 6128; fax: +49 941 944 6169.
*E-mail address:* christian.kirschneck@ukr.de (C. Kirschneck).

0940-9602/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.aanat.2013.08.003

onwards were included. A similar survey conducted by the authors of this article in 2013 showed a threefold increase of 545 studies in less than a decade, demonstrating the great demand in current orthodontic research for a reliable and precise rat model to measure orthodontic tooth movement.

Many studies on orthodontic tooth movement in rats have used rather unreliable, imprecise and non-physiological experimental designs that often do not conform to animal welfare guidelines. In addition, the methods for measuring tooth movement used so far are by no means perfect. These methods often solely rely on crude, undefined optometric measurements (Misawa-Kageyama et al., 2007; Rashidpour et al., 2012) or use two-dimensional radiological imaging (King et al., 1991; Ortega et al., 2012), which has the disadvantages of object superimposition and reduced reproducibility of identical undistorted images in consecutive measurements. Most studies also fail to exactly specify the landmarks used for measurement and the type of tooth movement measured (Ren et al., 2004). Furthermore many studies disregard confounding influences on orthodontic tooth movement and its measurement such as cranial growth that result in unreliable relations with regard to force, time and motion (King et al., 1991; Shirazi et al., 2001).

During the past fifteen years three-dimensional imaging systems such as cone beam computed tomography (CBCT) and X-ray microtomography have become important tools in orthodontic and anatomical research (Gonzales et al., 2009; Hashimoto et al., 2013; Radlanski et al., 1999; Sirisoontorn et al., 2011). These tools not only allow the analysis of tooth movement in general, but also the determination of the exact proportion of the different types of tooth movement (tipping, body movement and root torque) as well as their kinetics. Up to now, no system has been devised for analysing differentiated tooth movement that allows such measurements. Most studies have used three-dimensional imaging only for the measurement of the gap between the first (M1) and the second (M2) upper molars (Hashimoto et al., 2013; Sirisoontorn et al., 2011) or of the width of the periodontal ligament at various points (Gonzales et al., 2009). In addition, the effects of orthodontic force application on rat incisors have only been partially analysed (Cuoghi et al., 2013; Drevensek et al., 2009), although these incisors are often either moved orthodontically (Na et al., 2008; Olyaee et al., 2013) or used as an anchorage location for orthodontic appliances destined to move the rat molars orthodontically (Fujita et al., 2008; Karras et al., 2009).

Therefore, this study aims to (a) present a valid, reliable and physiological animal model and an experimental procedure for orthodontic tooth movement while simultaneously guaranteeing animal welfare, (b) introduce a reliable and precise optometric and cephalometric measurement system for orthodontic tooth movement in rats using optical (CCD microscope camera) and three-dimensional radiological imaging (cone beam computed tomography, CBCT), detailing the various types of tooth movement involved, (c) identify and analyse the influence of confounding parameters on orthodontic tooth movement and its measurement in the proposed rat model, (d) analyse the kinetics of orthodontic tooth movement achieved with this model, (e) analyse the effects of orthodontic forces on the upper incisors used as an anchorage, particularly the influence on the incisor eruption rate, (f) determine the exact individual contribution of the various types of tooth movement to overall tooth displacement using the proposed model and (g) further improvement on the limitations of previous studies.

## 2. Materials and methods

### 2.1. Experimental design and laboratory animals

A total of 24 male Outbred Wistar rats from Charles River Laboratories Inc. (Sulzfeld, Germany, Crl:WI) were randomly allocated to two groups of 12 animals each and received orthodontic treatment for two and four weeks, respectively. The animal experiments were conducted with the approval and permission of the Government of Upper Palatinate, Bavaria, Germany (approval ID: 54-2532.1-24/11) and in accordance with the German Animal Welfare Act. To prevent unnecessary animal suffering during the experiments, we specified humane endpoints and monitored the condition and weight of the animals on a daily basis (Stokes, 2002). The animals were kept in a conventional open-system S1 animal laboratory at the University of Regensburg, Germany. This laboratory had a temperature of 21 °C (SD 1 °C) and a relative humidity of 55% (SD 10%) in a noise-free environment. A 12 h alternating light and dark schedule was maintained, the automatically controlled light phase lasting from 7:00 am to 7:00 pm. The rats were kept in groups of four in transparent type IV-polycarbonate cages with metal grid tops in an animal holding cabinet. The animals were given distilled water in plastic bottles renewed twice a week and a standard rat and mouse maintenance diet (V1535, ssniff Spezialdiäten GmbH, Soest, Germany), both provided *ad libitum*. At the beginning of orthodontic treatment the solid, pellet-pressed chow was soaked in distilled water to mix it to mash, which was then placed on a tray within the cage. An acclimatisation period of four weeks between shipment and the beginning of the experiment resulted in a mean starting age of the rats of 61 days (SD 3 days) and a mean starting weight of 328 g (SD 28 g).

### 2.2. Orthodontic treatment

The animals were sedated with a combination of 6 mg xylazine and 90 mg ketamine per kg body weight, which was applied intraperitoneally as described by Jones (2012). We individually fixated the rats within a custom-made intervention apparatus (Fig. 1a/b) with their back on the surface of a wooden holding board coated in fluid-repellent, glossy white varnish. Two lateral metallic sliding bars were used to fix the bodies, while two further sliding bars were adjusted to the neck of the rats to keep their heads steady during orthodontic treatment. The head was bedded on a piece of polyurethane foam to prevent injuries during treatment, particularly to the eyes. We fixated the upper jaw with a transpalatinal bar between the upper incisors and the molars. The bar consisted of a stainless steel anatomical probe mounted to the board with tension-breaking orthodontic elastics (Fig. 1a). The lower jaw and tongue were retracted by a wire sling placed around the lower incisors and the tongue. This wire, which incorporated a closed coil as a tension breaker, was stretched until oral aperture was adequate and then fixated in this position to the top of the vertical beam of the equipment. The cheek on the intervention side (the left side of the animal, chosen to facilitate appliance fixation for a right-handed operator) was manually retracted with a dental mixing spatula, bent at the tip at a right angle, while the working hand of the researcher remained free for orthodontic manipulation. Sufficient illumination was provided by an LED light source mounted onto the adjustable arm of the equipment. The researcher carrying out orthodontic treatment used magnifying spectacles with a magnification of 2.5×. We modified a Sentalloy™ closed coil spring (ultra-light, number 10-000-26, Dentsply GAC Germany, Graefelfing) measuring 3 mm in length (without eyelets) by clipping the eyelets and by inserting two orthodontic stainless steel wires measuring 0.1 mm each directly into the coil (Fig. 1c). To avoid altering the effective length of the spring between the eyelets and thus the force applied, we inserted the wires prior to clipping.

Before orthodontic intervention we disinfected the oral cavity with chlorhexidine-drenched cotton pellets (Chlorhexamed® forte 0.2%, GlaxoSmithKline, Germany). We attached the spring to the cervix of the first upper molar in the left half of the upper jaw with a wire sling that encompassed the molar as well as the terminal