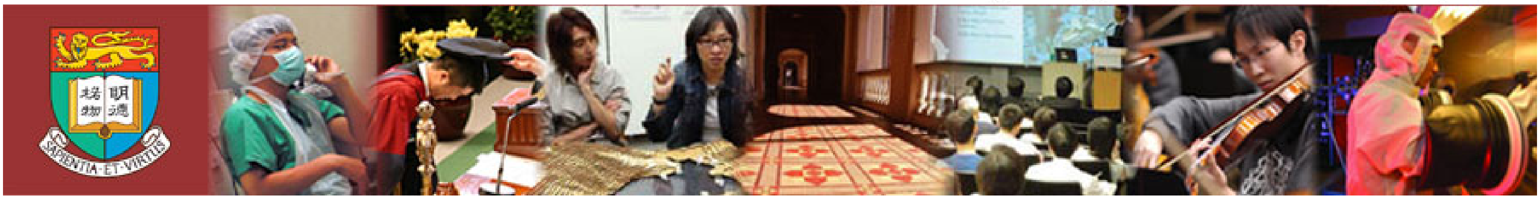


Title	A comparison between a conventional optical method and image-analysis for measuring the unimpeded eruption rate of the rat mandibular incisor
Author(s)	Lee, CK; Law, KT; King, NM; Rabie, ABM
Citation	Archives Of Oral Biology, 2002, v. 47 n. 7, p. 555-562
Issued Date	2002
URL	http://hdl.handle.net/10722/48488
Rights	Creative Commons: Attribution 3.0 Hong Kong License

A comparison of a conventional optical method with image analysis for measuring the unimpeded eruption rate of the mandibular rat incisor.

Running title: unimpeded eruption rate of rat incisor

Lee Chun Kei, Law Kwok Tung,

King Nigel Martyn*, Rabie Abou-Bakr Mahmoud

Faculty of Dentistry, The University of Hong Kong,

Prince Philip Dental Hospital, 34, Hospital Road, Hong Kong SAR.

* Corresponding author: Professor NM King

Fax number: 852 - 2559 3803

E-mail address: nmking@glink.net.

ABSTRACT

The measuring of impeded and unimpeded eruption rates are an integral part of most studies of the rat incisor. The most commonly used optical microscope method which has inherent weaknesses was compared in this study with an image analysis method to measure the eruption rate of the mandibular rat incisor. The study also evaluated the incisal edge as a reference point for the measurement of the unimpeded eruption rate and the frequency of shortening the mandibular incisor. The image analysis method was found to be a simple and reliable technique that could replace the optical method. There was no significant difference between the data as measured by the two methods. The Kappa coefficients for the two methods were similar at the 0.99 level. It was concluded that the shortening of the incisor for the measurement of the unimpeded eruption rate should be performed at least every 48 hours and the incisal edge of the shortened incisor can be used as the reference point for the measurement of the unimpeded eruption rate.

KEY WORDS

rat incisor, image analysis, unimpeded eruption rate

INTRODUCTION

Rats are frequently used in animal studies in preference to dogs and cats because they can be breed cheaply in large quantities (Berman and Massler 1958, Kirk et al 1989) and they can be handled relatively easily by a single operator (Mohammed and Schour 1955, Rowe 1967, Vongsavan et al 2000). The ability of the rat incisor to grow, calcify and erupt continuously during the life of the animal makes it an almost ideal model for the study of tissue interaction as, unlike other teeth, all stages of development are present within a single tooth, irrespective of the age of the animal (Shore et al 1992). Measuring the eruption rate of the incisor, has been said to be, an integral part of expanding the knowledge of the process of tooth eruption (Taverne 1991). Moreover, the eruption rate of the rat incisor is often utilized as a convenient means of assessing the effects, on the development of the tooth, of a variety of stimuli, such as nutrients, hormones and toxic substances (Michaeli and Greulich 1972, Burn-Murdoch 1993, Gerlach et al 2000).

There are several established methods for measuring the eruption rate of the rat incisor; some involve the taking of measurements over a period of several days (Tsuruta et al 1974), or even weeks (Burn-Murdoch 1995, Risnes et al 1995); another way is to use non-contacting displacement detectors over a period of hours

(Chiba et al 1995, Ohyama and Yamaguchi 1999). For the taking of measurements over a long time period, the most commonly used method is the optical microscope with a graticule in the eyepiece which produces a direct measurement; other methods include the use of calipers connected to a digital voltmeter (Michaeli et al 1975, Pitaru et al 1976, Weinreb et al 1985, Michaeli et al 1985, Steigman et al 1988, Brin et al 1990), radiographs (Ness 1954, 1956, 1965, Adams and Main 1962, Main and Adams 1966, Chiba et al 1968, Lavelle 1968, 1969) and photographs (Chiba et al 1973, 1980, 1981, Tsuruta et al 1974, Robins and Spicer 1981, Chiba and Ohshima 1985). However, none of the currently used methods is entirely satisfactory. Hence, the objective of this study was to develop a method that was simple and quick to use, which avoided the use of irradiation; did not introduce unnecessary errors as a consequence of processing photographic films, and provided a permanent record so results could be re-checked. In addition, it was desirable that the method could be performed at leisure with a high level of sensitivity and would overcome the problem of the small, but rapid, movements of an anaesthetized rat during breathing.

Repeated shortening of the incisal edge of the rat incisor produces a marked increase in the eruption rate of the tooth, a phenomenon which was first reported by Wetzel in

1927 (cited by Taylor and Butcher 1951). In 1957, Bryer coined the term "unimpeded" eruption to indicate that sufficient coronal tooth substance had been removed to prevent "special attritional activity" of the incisors against each other. However, a doubling of the eruption rate returns the incisor into occlusion within a short period of time. If the unimpeded incisor is not out of occlusion, due to an inadequate degree or frequency of shortening, wear facets will be produced on the incisal edge. Measurement errors occur if the incisal edge, rather than a cut mark on the tooth, is used as a reference point; a phenomenon that has been reported in a number of previous studies (Bryer 1957, Ness 1965, Sessle 1966, Berkovitz 1974, Burn-Murdoch 1988, Gerlach et al 2000). Therefore, the suitability of using the incisal edge of an unimpeded incisor as the reference point in the measurement of unimpeded eruption rate was investigated in this study; which also sought to see whether there was a difference in the unimpeded eruption rate when the incisor was shortened at 2-day and 3-day intervals.

MATERIALS AND METHODS

Thirty-four Sprague-Dawley rats, seven to eight weeks old, with an average weight of 191.2g (175.6g-206.6g) were divided into two groups. Twelve rats were assigned to the control group and 22 rats to the experimental group. All of the rats were placed in a windowless room which was illuminated artificially from 0700 to 1900 hours and was otherwise dark. The temperature and humidity were controlled to be 21^0C ± 1^0C and from 55% to 73% respectively. The rats were caged in groups of two to three, fed on laboratory autoclavable pellet rodent diet (PMI Nutrition International Inc.) and able to take water *ad libitum*.

Operative procedure

Every Monday, Wednesday and Friday between 0900 and 1400 hours, the rats were weighed and then anaesthetised with an intra-peritoneal injection of Ketamine (67mg/kg) and Xylazine (6.7mg/kg). The experiment was always started promptly at 0900 hours. The rats were routinely anaesthetised in the same sequence in order to ensure the accuracy of the 48hrs or 72hrs time periods. For the animals in the control and experimental groups, marks were cut below the level of the interdental papilla on both mandibular incisors with a diamond disc (Horico H362F 080, 0.18mm thick) while, for the animals in the experimental group, the mandibular right incisor was shortened with the same type of diamond disc at the level of the interdental papilla (Figure 1). The experimental procedure was approved by the Committee on the Use of Live Animals in Teaching and Research, University of Hong Kong.

Recording method

The length of the incisor was measured using the graticule in the eyepiece of a stereomicroscope (Nikon SMZ-10). Simultaneously, the image was captured by a colour video camera (JVC TK1080E) attached to the microscope. The length of the tooth was then measured with the programme, NIH Image Version 1.61. At the start of every session, the graticule of the eyepiece was calibrated with a 10mm micrometer with one scale equivalent to 100µm. At the same time, the image was captured by the video camera and the 10mm scale was calibrated. The calibration procedure was performed twice and if the readings deviated by more than 0.1%, it was re-calibrated.

For the control group, the lowest point of the labial gingiva was used as the reference point on the respective incisors and it was always placed at the “zero” point of the graticule. Four readings were taken for each rat (Figure 1a). The distance from the gingiva to the cut mark, and the distance from the gingiva to the incisal edge of both incisors were measured. Due to the thickness of the diamond disc, the mid-point of the cut mark was used for all of the measurements. For the experimental group, the readings for both the right unimpeded incisor and the left unshortened incisor were taken with the reference mark being the lowest point of the labial gingiva on the left unshortened incisor (Figure 1b). This reference point was used because it has been considered by other workers to be more stable (Aladdin and Burn-Murdoch 1985b). The direct optical readings were always taken by the same operator (CK) and then recorded by an assistant.

After capturing the image using the video camera, another operator (KT) took the same four readings for the control group of teeth. For the experimental group, a line was first drawn between the two incisors and the lowest point of the gingiva of the left unshortened incisor was chosen as the reference point. A virtual line was then created by the computer perpendicular to the line which passed through the reference point. The four readings were taken from this line along the midline of the incisor to the cut mark, or the incisal edge, as shown in Figure 2.

Eruption rate measurement

In the subsequent sessions, i.e. two or three days later, the cut mark had moved incisally due to the continuous eruption of the incisor. For each rat, four readings were again recorded. The eruption rate was then calculated as being the distance that the cut mark had travelled per day. For the unimpeded tooth in the experimental group, the distance that the incisal edge had travelled per day was also calculated. In this way, the eruption rates calculated from the two different reference points, could be compared in order to assess the validity of using the incisal edge as a reference point for measuring the unimpeded eruption rate.

For the control group, a new mark was cut below the level of the interdental papilla and the new readings were taken. For the experimental group, the right incisor was again shortened to the level of the interdental papilla and new readings were taken. The same operative procedures were performed at every data collection session during the three-week experimental period. Therefore, at every session except the first and the last, the length of the unshortened left incisor in both the control and experimental groups were measured twice, once before and once after cutting a new mark. If the reading from the direct optical measurement differed by more than 0.3mm, the investigator was informed by the recording assistant so that the measurement could be rechecked. However, once the reading was measured by the image analysis method, it was stored in the computer and was not rechecked. These repeat readings were used to test the reliability of the two different measuring methods.

As the experiments were carried out on Monday, Wednesday and Friday for a period of 3 weeks, the eruption rate was measured after two days on six occasions (Day 2, 4, 9, 11, 16, 18) and after three days on three occasions (Day 7, 14, 21). One way ANOVA was used to test for any difference in the mean of the unimpeded eruption rate on the nine occasions. If the p-value was <0.05, then the Student-Newman-Keuls test was used to test for any homogenecity for the 2-day or 3-day periods. There were two different unimpeded eruption rates, one measured from the incisal edge and one from the cut mark in each of the two measuring methods. Therefore, a total of four data sets for the unimpeded eruption rate were obtained for the one way ANOVA test.

RESULTS

All of the 34 rats survived the 3-week experimental period. There were no significant differences in the weights of the rats between the two groups on any of the 10 measurement occasions during the study. Although the average weight gain for the experimental group was 11% less than the control group, it was not statistically significant.

Measuring method

In the testing of the reliability of the two methods, a total of 272 pairs of measurements of the length of the left incisor in both groups were obtained. The data for the first and last visits were not used because only one reading was taken on those two visits. The Kappa coefficient for the optical method was 0.992 while that for the image analysis method was 0.991. Comparison of the variances of the two groups of length measurements for the left incisor from the two methods with Levene's test indicated no significant difference between the first measurements, the repeated second measurements, or the average of the two measurements (Table 1).

When the eruption rates of the right incisors in the control group obtained by the two measuring methods were compared, there was a significant difference on Day 4 only, while there was no significant difference in the eruption rates of the left incisor in the control group obtained by the two methods on all of the recording occasions. For the experimental group, there was also no significant difference in the impeded eruption rate and the unimpeded eruption rate (using the cut mark as the reference point for the measurements) obtained by the two methods on all of the recording occasions. If the unimpeded eruption rate was measured by using the incisal edge as the reference point, there was a significant difference between the two measuring methods for Day 2 only. All of the results are displayed in Table 2.

Eruption rate measurement

The unimpeded eruption rates, measured with the cut mark as the reference point by both the optical method and the image analysis method, are shown in Figure 3. The unimpeded eruption rate was measured by the two recording methods using two different reference points; incisal edge and cut mark. Therefore, a total of four different sets of unimpeded eruption rate readings were obtained. For each of the four sets of unimpeded eruption rates, one way ANOVA test showed significant differences ($p<0.001$) between the nine occasions. If the Student-Newman-Keuls test was used to test the homogenecity for the nine occasions, the data for Day 7, 9, 14, 21 could always be grouped into a subset for all the four sets of unimpeded eruption rates (Table 3).

When the mean unimpeded eruption rate, calculated using the incisal edge as the reference point was compared to that using the cut mark as the reference point for each of the recording occasions, there was no statistically significant difference in the image analysis method (Figure 4); the result was apparently the same for the optical method.

DISCUSSION

During the 3-week experimental period, there was no statistically significant difference in the weight of the control and experimental rats in spite of a slightly lower weight gain by the animals in the experimental group. This finding was consistent with that of previous studies (Steigman et al 1989, Brin et al 1990). However, in some studies, when the rats had the mandibular incisors unimpeded, the weight gain was significantly less than that in the normal functioning group (Wells et al 1959, Sarnat and Sciaky 1965, Chiba et al 1981). The report of Wells and Munson (1959) even showed a negative relationship between the weight gain and the number of amputations. The decrease in weight gain has been attributed to the diminished feeding efficiency and food intake (Houssay and Alvarez Ugarte 1969, Chiba et al 1981). In some other studies, it was found that the rats actually had a weight loss after repeated shortening of the incisors (Houssay and Ugarte 1969, Yagil and Barka 1986). However, it has been shown that the weight loss was not associated with any significant changes in the unimpeded eruption rate of the incisors (Bryer 1957, Adatia and Berkovitz 1981, Moxham and Berkovitz 1983). This is because the growth and eruption of the teeth are much less vulnerable than the growth of the body (Orban 1927, Weinreb et al 1967). Therefore, the slightly lower weight gain of the rats in the experimental group could not be expected to have any untoward effect on the results of this study.

When the eruption rate obtained by the optical method and the image analysis method were compared, there was a significant difference only for the right incisor of the control group on Day 4 and for the unimpeded incisor of the experimental group (using the incisal edge as the reference point) on Day 2 (Table 2). There was no significant difference when the eruption rate obtained by the optical method and the image analysis method were compared for the left incisor of the control group, the unimpeded incisor of the experimental group (using the cut mark as the reference point) or the right unshortened incisor of the experimental group (Table 2). Therefore, it can be concluded that, in general, the two measuring methods used in this study, to measure the eruption rate, were equally as effective.

The reliability of both methods was checked by retaking the measurements of the length of the left incisor and this produced a total of 272 sets of data. A comparison of the first readings in each of the two methods by testing the variances did not reveal a statistical significant difference. Neither were there any statistical differences between the second readings, nor the average of the two sets of readings. The Kappa coefficients for the optical method and the image analysis method were

0.992 and 0.991 respectively which were considered to be "very good". However, the experimental conditions for the optical method were less stringent than for the image analysis method because, if the repeated reading had a difference of greater than 0.3mm, it was rechecked in order to avoid introducing any human recording errors, while, in the image analysis method, once a reading had been made, it was stored. This approach may be criticized because the observers' memory can be expected to have had some influence over the outcome of the direct measurement method.

The present results clearly demonstrated that the image analysis method offers several distinct advantages over the optical method. A large sample size is always necessary to improve the statistical power of any study but the lengthy process involved in taking readings with the microscope will inevitably create some human errors. During the repeat measurements of the length of the left incisor when testing the reliability of the two methods, the author encountered, on several occasions, erroneous measurements with a difference of greater than 0.3mm, which was frequently due to simply misreading of the graticule. Therefore, this phenomenon can be expected to occur with readings in any experiment. In data from the optical measurements, in the present study, eleven (0.6%) erroneous readings, out of a total of 1836 readings, were found; which adversely affected the results by indicating an apparent negative eruption rate, or negative attrition rate, or by just producing an extreme reading. Therefore, in the course of this experiment, modifications were made to the eleven erroneous readings by using the image analysis readings, or re-measuring from the captured image. The permanent records of the images obtained from the image analysis method offer the possibility to recheck any aberrant readings. Moreover, the measurements can be made at the investigators' leisure, which helps to reduce stress and, in turn, human error. It also allows the taking of multiple readings for statistical analysis of for instance the inter-examiner or intra-examiner reproducibility. As Ness (1964) pointed out, the high breathing rate of the rat can cause difficulties when direct recording are taken under the microscope, but this can be easily overcome by using the image analysis method.

When assessing the unimpeded eruption rate, the labial gingiva of the adjacent unshortened incisor was used as the reference point, which was in accordance with many other studies (Aladdin and Burn-Murdoch 1985a, 1985b, Burn-Murdoch 1988, 1990). This was because, according to several investigators, the gingiva of the unimpeded incisor may overgrow and become friable when the incisor is shortened regularly (Aladdin and Burn-Murdoch 1985b, Skobe et al 1993); this phenomenon also happened in the present study. This can create some difficulties, or even errors when making the measurements for the unimpeded incisor because the cut mark, or incisal edge of the unimpeded incisor and the labial gingiva of the adjacent impeded incisor do not always lie on a straight line of the graticule in the eyepiece. Moreover, one unit of scale of the graticule was equal to 0.1mm; therefore, the operator could be expected to measure to an accuracy of only 0.05mm. The image analysis method overcomes these problems because the accuracy of the measurement depends on the size of the pixels and the sensitivity of the computer mouse, so the level of accuracy can be at least up to 0.01mm. Many problems encountered in making the measurements are easily solved by the computer software NIH Image Version 1.61. The operator just needs to draw a line between the two incisors and identify the labial gingiva of the unimpeded incisor. A perpendicular line which passes through the labial gingiva reference points is then automatically drawn. Subsequently, the distance of the cut mark, or the incisal edge of the unimpeded incisor to this created line can be measured easily and without errors (Figure 2). Although the image analysis method clearly offers a lot of advantages over the optical method, it does have some minor limitations. For example, the quality of the image is never as good as the image seen through the eyepiece because there is invariably some loss of quality of the image given by the video camera and the computer. Nevertheless, the use of a high quality video camera can easily overcome this problem.

The unimpeded eruption rate is intended to be measured by keeping the incisor free of occlusal stress. However, the increased eruption rate after shortening causes the incisor to move back into occlusion within a short time. Therefore, the degree of shortening, and the frequency of shortening are essential elements in the success of this type of study. In the present investigation, the incisors were shortened to the level of the interdental papilla, which was the level used in most studies (Lavelle 1968, Tsuruta et al 1974, Ohshima and Chiba 1981, Weinreb et al 1985, Yagil and Barka 1986, Sato et al 1996, Gerlach et al 200). It was decided to shorten the incisors three times a week, in accordance with many other investigators (Chiba et al 1968, Berkovitz 1974, Weinreb et al 1985, Steigman et al 1988, Shore et al 1992, Kirkham et al 1993). As the eruption rate of the incisor is measured by the distance the incisor erupts in a given period of time, consideration needs to be given not only to the number of days, but also to the number of hours that elapse between the shortening procedures. In the experiment by Risnes and co-workers (1995), they measured the eruption rate on Monday morning, Wednesday at noon and Friday afternoon. Therefore, the quoted eruption rate was theoretically incorrect because only the number of days was taken into consideration. Burn-Murdoch carried out the experiments every 46-50 hours (Burn-Murdoch 1985b) and every 48 hours with no time interval being different by a factor of more than 10% (Burn-Murdoch 1988). In accordance with Burn-Murdoch (1988), the experimental protocol in the current study was commenced at precisely 0900hrs and then adhered to the same sequence of anaesthetizing the rats at every session in order to measure the eruption rate every 48 hours or 72 hours.

Measurements were obtained for the unimpeded eruption rate on six occasions at 2-day intervals and on three occasions at 3-day intervals. The graph in Figure 3 shows three troughs which corresponded to Day 7, 14 and 21 when the incisors were shortened after these 3-day intervals. One way ANOVA test showed that there was a significant difference ($p<0.001$) in the unimpeded eruption rate as measured on nine occasions. The Student-Newman-Keuls test showed that mainly measurements on Day 7, 14, 21 (3-day interval) and Day 2, 9 (2-day interval) formed a homogenous subset. The reason why the unimpeded eruption rate was low at Day 2 is that the unimpeded eruption rate has been shown to rise initially until it reaches a steady level four days after shortening (Michaeli et al 1974, 1975, Risnes et al 1995). However, this does not fully explain the reason why the unimpeded eruption rate at Day 9 was also as low as on Day 7, 14 and 21. The above findings suggest that the unimpeded eruption rate caused by shortening after a 3-day interval produces a lower reading than when the shortening is performed after a 2-day interval.

Taylor and Butcher (1951) mentioned that, because of the accelerated eruption of the unimpeded tooth and the increased attrition of its adjacent impeded tooth, the shortening had to be repeated frequently in order to prevent re-establishment of the occlusal contact. In spite of this knowledge being in the published literature, the frequency of shortening varies form study to study within the range from every two days to twice a week. If the shortening is performed at irregular intervals, the eruption rate tends to drop when it is preceded by a long period without shortening; this is probably due to the return of the tooth into occlusion (Taylor and Butcher 1951). Even though it is satisfactory to shorten the rabbit incisor twice a week because of the tooth’s greater length (Ness 1956), the shorter length of the rat incisor means that the tooth is again in occlusion three or four days after cutting of the crown (Orsós and Bartha 1956, Bryer 1957). Tsuruta and co-workers (1974) explained the decrease in the unimpeded eruption rate as being the result of incomplete elimination of occlusal contact, while Burn-Murdoch (1993, 1995) indicated that, when shortening the incisor every two days, wear facets could be seen in the smaller rats of weights 305g-385g, but not in the older rats with weights of 415g-470g. This age factor may be of significance when planning a study to at least ensure consistency, by choosing rats within a specific weight range.

Thus, it can be seen that an optimal frequency and level of incisor shortening is required in order to keep the incisor unimpeded and thus free of occlusal stress. The absence of wear facets on the cut end of the tooth is said to be a useful clinical indication that a tooth is not in occlusion. However, Burn-Murdoch (1995) who shortened the incisor to the gingival margin, at 2-days intervals, and still found wear facets on the unimpeded incisor, concluded that this small amount of wear probably had a negligible effect on the eruption rate and that a length difference, between the unimpeded incisor and its adjacent incisor, of greater than 0.5mm could render the incisor fully unimpeded (Burn-Murdoch 1999). Therefore, provided there is only a small amount of wear, it may not actually produce significant errors in the unimpeded eruption rate.

In the present study using the optical method, five rats on Day 7, one rat on Day 9 and three rats on Day 14 had their shortened right unimpeded incisor grow longer than the adjacent incisors. Similar results were found for the image analysis method except that none of the rats on Day 5 had a longer unimpeded incisor. The differences in the length of the two incisors, as calculated for both methods are shown in Table 4. From these findings, it can be seen that after all of the 3-day intervals which occurred at Day 7, Day 14, Day 21, the differences in the length of the two incisors was less than 0.5mm, because three days after shortening, the unimpeded incisor had almost grown back to the occlusal level. Therefore, the shortened incisors were not free of occlusal stress anymore; hence, the unimpeded eruption rate was reduced.

Although wear facets of greater than 0.5mm may not affect the unimpeded eruption rate, they do affect the measurements, especially if the incisal end which will be removed by attrition is used as the reference point for measurements. In numerous studies (Bryer 1957, Sessle 1966, Berkovitz 1974, Burn-Murdoch 1988, Gerlach et al 2000), the incisal edge of the unimpeded incisor was used as a reference point for the assessment of unimpeded eruption rate. Even in his radiographic study, Ness (1965) used the incisal edge of the unimpeded teeth as the reference point. This probably does not produce a true value if tissue is removed from the incisal edge by attrition. Michaeli and Weinreb (1968) were the first investigators to cut a mark on the unimpeded incisor and then to use it as the reference point. Based on these observations, it was decided that in this study, the unimpeded eruption rate was to be estimated by using both the incisal edge and the cut mark as reference points. It was subsequently found that there was no significant difference in the eruption rate as measured by using the two different reference points. This may be because the enamel is only present on the buccal side of the incisor and that the attrition is mainly on the lingual side (Risnes et al 1995), which will consequently not affect the measurements.

In summary, the image analysis method was found to be a simple, reliable and accurate method for measuring the eruption rate of the rat incisor that could replace the commonly used optical method. For the shortening of the incisor to provide a truly unimpeded eruption rate, the incisor needs to be shortened at least every 48 hours in 7-8 weeks old rats. In addition, the incisal edge of the shortened tooth can be used as the reference point for taking measurements.

ACKNOWLEDGEMENTS

We express our sincere gratitude to Mr. Shadow Yeung for his generous help in the statistical analysis. This study was supported by CRC grant 10203279.07840.08004.323.01, from the University of Hong Kong.

REFERENCES

Adams, D., Main, J.H.P., 1962. A radiographic method of measuring unimpeded eruption rates in the rat incisor. Journal of Physiology 160, 1P-2P.

Adatia, A.K., Berkovitz, B.K.B., 1981. The effects of cyclophosphamide on eruption of the continuously growing mandibular incisor of the rat. Archives of Oral Biology 26, 607-613.

Aladdin, Q.I., Burn-Murdoch, R.A., 1985a. The effect of procedures intended to alter the interstitial fluid pressure in the sockets of resected rat incisors on their eruption rate. Archives of Oral Biology 30, 525-530.

Aladdin, Q.I., Burn-Murdoch, R.A., 1985b. Techniques for choosing reference points from which to measure eruption rates of rat incisors. Archives of Oral Biology 30, 531-537.

Berkovitz, B.K.B., 1974. Effect of fluoride on eruption rates of rat incisors. Journal of Dental Research 53, 334-337.

Berman, D.S., Massler, M., 1958. Experimental pulpotomies in rat molars. Journal of Dental Research 37, 229-242.

Brin, I., Steigman, S., Michaeli, Y., 1990. Effect of occlusal functional forces on incisor socket morphology and location in rat mandible. Anatomical Record 226, 367-372.

Bryer, L.W., 1957. An experimental evaluation of the physiology of tooth eruption. International Dental Journal 7, 432-478.

Burn-Murdoch, R.A., 1988. The effects of corticosteriods and cyclophosphamide on the eruption of resected incisor teeth in the rat. Archives of Oral Biology 33, 661-667.

Burn-Murdoch, R.A., 1990. The effect of cycolphosphamide on the eruption of impeded and resected incisors in rats. Archives of Oral Biology 35, 801-806.

Burn-Murdoch, R.A., 1993. The effect of the consistency of the diet on eruption rates and lengths of incisor teeth in rats. Archives of Oral Biology 40, 699-706.

Burn-Murdoch, R.A., 1995. The effect of shortening incisor teeth on the eruption rates and lengths of the other incisors in the rat. Archives of Oral Biology 40, 467-471.

Burn-Murdoch, R.A., 1999. The length and eruption rates of incisor teeth in rats after one or more of them had been unimpeded. European Journal of Orthodontics 21, 49-56.

Chiba, M., Ohshima, S., 1985. Effects of colchicine and hydrocortisone on unimpeded eruption rates of root-resected mandibular incisors of rats. Archives of Oral Biology 30, 147-153.

Chiba, M., Komatsu, K., Yamaguchi, S., 1995. Axial movements of rat mandibular incisors measured under artificial respiration with halothane anaesthesia. Archives of Oral Biology 40, 269-274.

Chiba, M., Kuroda, T., Oshshima, S., 1981. Effects of adrenocorticoids on impeded and unimpeded eruption rates and on the mechanical properties of the periodontium in the rat mandibular incisor. Archives of Oral Biology 26, 577-583.

Chiba, M., Narraway, J.M., Ness, A.R., 1968. Impeded and unimpeded eruption of the mandibular incisor of the adult male rat and its stoppage by demecolcine. Journal of Dental Research 47, 986.

Chiba, M., Takizawa, K., Ohshima, S., 1980. Dose-response effects of colchicine and vinblastine on unimpeded eruption rates of the rat mandibular incisor. Archives of Oral Biology 25, 115-119.

Chiba, M., Tsuruta, M., Eto, K., 1973. A photographic method of measuring eruption rates of rat mandibular incisors. Archives of Oral Biology 18, 1003-1010.

Gerlach, R.F., Toledo, D.B., Novaes, P.D., Merzel, J., Line, S.R.P., 2000. The effect of lead on the eruption rates of incisor teeth in rats. Archives of Oral Biology 45, 951-955.

Houssay, A.B., Alvarez Ugarte, C.A., 1969. On the mechanism of the hypertrophy of the retrolingual glands of the rat induced by incisor amputations. Archives of Oral Biology 14, 63-69.

Kirk, E.E.J., Lim, K.C., Khan, M.O.G., 1989. A comparison of dentinogenesis on pulp capping with calcium hydroxide in paste and cement form. Oral Surgery, Oral Medicine, Oral Pathology 68, 210-219.

Kirkham, J., Robinson, C., Phull, J.K., Shore, R.C., Moxham, B.J., Berkovitz, B.K.B., 1993. The effect of rate of eruption on periodontal ligament glycosylaminoglycan content and enamel formation in the rat incisor. Cell and Tissue Research 274, 413-419.

Lavelle, C.L.B., 1968. Effect of age on the rate of regrowth of the incisor teeth of the rat. Journal of Dental Research 47, 836.

Lavelle, C.L.B., 1969. The effect of age on the eruption rate of the incisor teeth of the rat (Rattus norvegius). Journal of Anatomy 104, 109-115.

Main, J.H.P., Adams, D., 1965. Measurement of the rate of eruption of the rat incisor. Archives of Oral Biology 10, 999-1008.

Michaeli, Y., Greulich, R.C., 1972. A three dimensional representation of the odontogenic epithelium of the rat incisor. Anatomic Records 174, 389-398.

Michaeli, Y., Weinreb, M.M., 1968. Role of attrition and occlusal contact in the physiology of the rat incisor: III. Prevention of attrition and occlusal contact in the nonarticulating incisor. Journal of Dental Research 47, 633-640.

Michaeli, Y., Weinreb, M.M., Zajicek, G., 1975. Role of attrition and occlusal contact in the physiology of the rat incisor: VIII. Tooth length and occlusal plane as regulating factors of eruption and attrition rates. Journal of Dental Research 53, 1215-1218.

Michaeli, Y., Pitaru, S., Zajicek, G., Weinreb, M.M., 1975. Role of attrition and occlusal contact in the physiology of the rat incisor: IX. Impeded and unimpeded eruption in lathyritic rats. Journal of Dental Research 54, 891-896.

Mohammed, C.I., Schour, I., 1955. Experimental cavity preparations in the incisor of the rat. Journal of Dental Research 34, 608-620.

Moxham, B.J., Berkovitz, B.K.B., 1983. Interactions between thyroxine, hydrocortisone and cyclophosphamide in their effects on the eruption of the rat mandibular incisor. Archives of Oral Biology 28, 1083-1087.

Ness, A.R., 1954. Measuring the continuous eruption of the rabbit mandibular incisor. Journal of Physiology 124, 13P-15P.

Ness, A.R., 1956. The response of the rabbit mandibular incisor to experimental shortening and to the prevention of its eruption. Proceedings of the Royal Society of London. Series B, Biological Sciences 146, 129-154.

Ness, A.R., 1964. Movement and forces in tooth eruption. Advances in Oral Biology 1, 33-75.

Ness, A.R., 1965. Eruption rates of impeded and unimpeded mandibular incisors of the adult laboratory mouse. Archives of Oral Biology 10, 439-451.

Ohshima, S., Chiba, M., 1981. Changes in mitotic activity in the basal proliferative tissues after experimental shortening of the rat incisor. Archives of Oral Biology 26, 533-535.

Ohyama, N., Yamaguchi, S., 1999. Effects of phenylephrine and prazosin on axial movement of the rat incisor and arterial blood pressure. Japan Journal of Pharmacology. 80, 271-274.

Orban, B., 1927. Nutrition and teeth. Journal of American Dental Association 14, 1619-1630.

Pitaru, S., Michaeli, Y., Zajicek, G., Weinreb, M.M., 1976. Role of attrition and occlusal contact in the physiology of the rat incisor: X. The part played by the periodontal ligament in the eruptive process. Journal of Dental Research 55, 819-824.

Risnes, S., Septier, D., Goldberg, M., 1995. Accelerated eruption of rat lower incisor. Relationship between impeded and unimpeded eruption rates, rate of attrition, tooth length and production of dentin and enamel. Connective Tissue Research 32, 183-189.

Robins, M.W., Spicer, R.M., 1981. The effect of halothane anaesthesia on the spontaneous movements of the mandibular incisor of the rat measured over periods of hours. Journal of Physiology 313, 44P.

Rowe, A.H.R., 1967. Reaction of the rat molar pulp to various materials. British Dental Journal 122, 291-300.

Sarnat, H., Sciaky, I., 1965. Experimental lathyrism in rats: effects of removing incisal stress. Periodontics 3, 128-134.

Sato, K., Hattori, M., Aoba, T., 1996. Disturbed enamel mineralization in a rat incisor model. Advanced Dental Research 10, 216-224.

Sessle, B.J., 1966. Attrition and eruption rates of the rat lower incisor. Journal of Dental Research 45, 1571.

Shore, R.C., Kolokuris, I., Robinson, C., Kirkham, J., 1992. Immunohistochemical investigation of epidermal growth factor receptor expression during periods of accelerated rat incisor eruption. Archives of Oral Biology 37, 389-393.

Skobe, Z., Heeley, J.D., Dobeck, J.M., Prostak, K.S., Maravelis, L., Stern, D.N., 1993. Comparison of rates of enamel synthesis in impeded and unimpeded rat incisors. Journal of Dental Research 72, 46-50.

Steigman, S., Barad A, Michaeli, Y., 1988. The effect of load duration on long-term recovery of the eruptive function in the rat incisor. American Journal of Orthodontics and Dentofacial Orthopaedics 93, 310-314.

Steigman, S., Michaeli, Y., Yitzhaki, M., Weinreb, M., 1989. A three-dimensional evaluation of the effects of functional occlusal forces on the morphology of dental and periodontal tissues of the rat incisor. Journal of Dental Research 68, 1269-1274.

Taverne, A. A.R., 1991. Reliability of eruption rate measurements of the rat incisor. Australian Orthodontic Journal 12, 33-36.

Taylor, A.C., Butcher, E.O., 1951. The regulation of eruption rate in the incisor teeth of the white rat. Journal of Experimental Zoology 117, 165-188.

Tsuruta, M., Eto, K., Chiba, M., 1974. Effect of daily or 4-hourly administrations of lathyrogens on the eruption rates of impeded and unimpeded mandibular incisors of rats. Archives of Oral Biology 19, 1221-1226.

Vongsavan, K., Vongsavan, N., Matthews, B., 2000. The permeability of the dentine and other tissues that are exposed at the tip of a rat incisor. Archives of Oral Biology 45, 927-930.

Weinreb, M.M., Assif, D., Michaeli, Y., 1967. Role of attrition in the physiology of the rat incisor: I. The relative value of different components of attrition and their effect on eruption. Journal of Dental Research 46, 527-531.

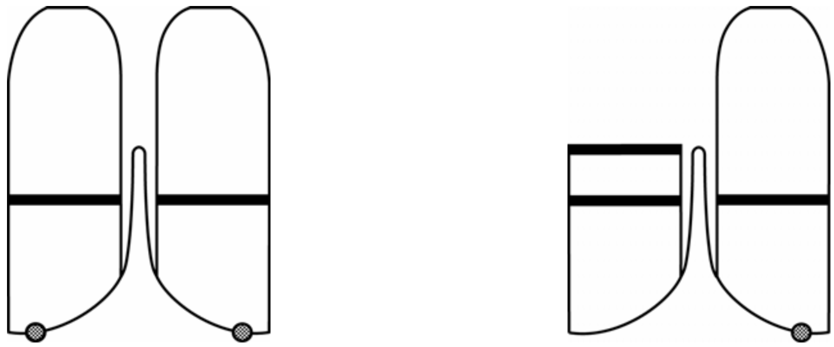
Weinreb, M., Steigman, S., Zajicek, G., Michaeli, Y., 1985. Odontoblast turnover in the impeded and unimpeded rat incisor derived from computerized histomorphometry. Anatomical Record 211, 218-225.

Wells, H., Zackin, J., Goldhaber, P., Munson, P.L., 1959. Increase in weight of the submandibular salivary glands following periodic amputation of the erupted portion of the incisor teeth. American Journal of Physiology 196, 827-830.

Yagil, C., Barka, T., 1986. Induction of a specific (LM) protein in the submandibular gland of the rat by repeated amputation of the lower incisor teeth. American Journal of Anatomy 177, 513-518.

VITAE

Dr Lee is studying for the Master of Dental Surgery in Paediatric Dentistry. Dr. Law is studying the Advanced Diploma in Paediatric Dentistry. Dr Rabie is an Associate Professor in Orthodontics and Director of Hard Tissue Research. Professor King is Professor in Paediatric Dentistry, Faculty of Dentistry, The University of Hong Kong. Dr Lee graduated with BDS from The University of Hong Kong and has obtained Membership of the Faculty of the General Dental Practitioners (UK), Fellowship of the Royal Australasian College of Dental Surgeons, and Membership in Paediatric Dentistry of the Royal College of Surgeons of Edinburgh. Dr. Law graduated with DDS from the Taipei Medical College and obtained his Master of Dental Surgery in Paediatric Dentistry from The University of Hong Kong. Dr Rabie received his orthodontic (Cert Ortho) and research training leading to a PhD degree at Northwestern University, Chicago, USA and is a Fellow of Hong Kong Academy of Medicine. Professor King holds BDS and MSc degrees with honours from The University of London and a PhD from The University of Hong Kong and is a Fellow of Hong Kong Academy of Medicine and a Fellow of the College of Dental Surgeons of Hong Kong.



(a) (b)

Figure 1. The reference points, level of putting cut marks and shortening of the rat incisor for the measurements. (a) For the control group, marks were cut below the interdental papilla level. The reference point was taken to be the lowest point of the labial gingiva on the respective tooth. (b) For the experimental group, the right incisor was shortened at the interdental papilla and a mark was cut below it. The reference point for both incisors was taken to be the lowest point of the labial gingiva on the left unshortened incisor.

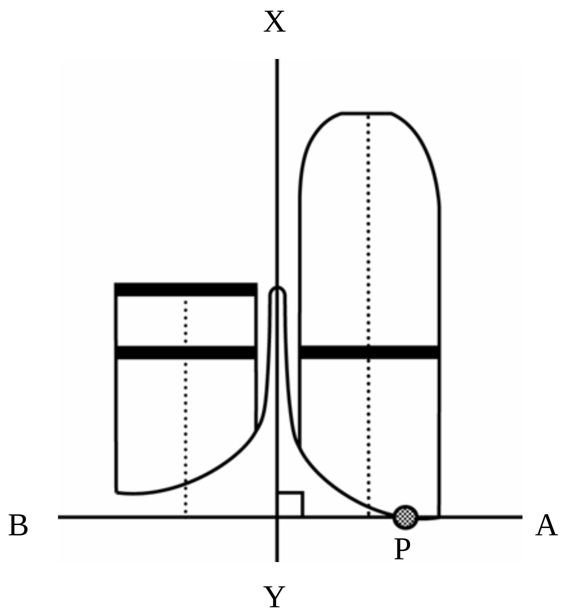


Figure 2. Length measurements in the image analysis method. With the aid of the computer software (NIH 1.61) in the image analysis method, a vertical line XY was manually drawn bisecting the two incisors, subsequently, a perpendicular line AB was created by the computer to pass through the chosen ginigval reference point P. The lengths of the incisors were then measured along the middle of the tooth to this created line.

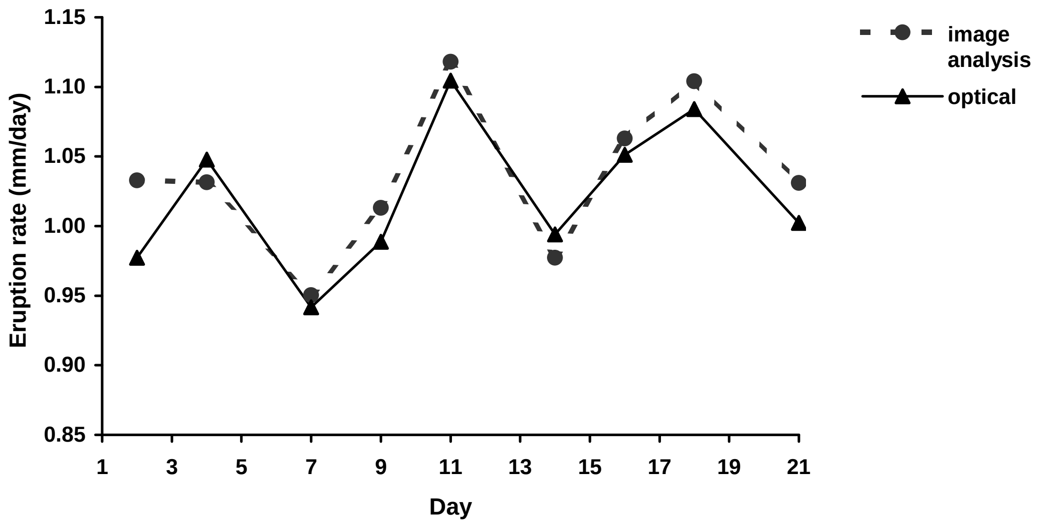


Figure 3. The unimpeded eruption rate measured by the optical and image analysis methods when cut marks were used as the reference point.

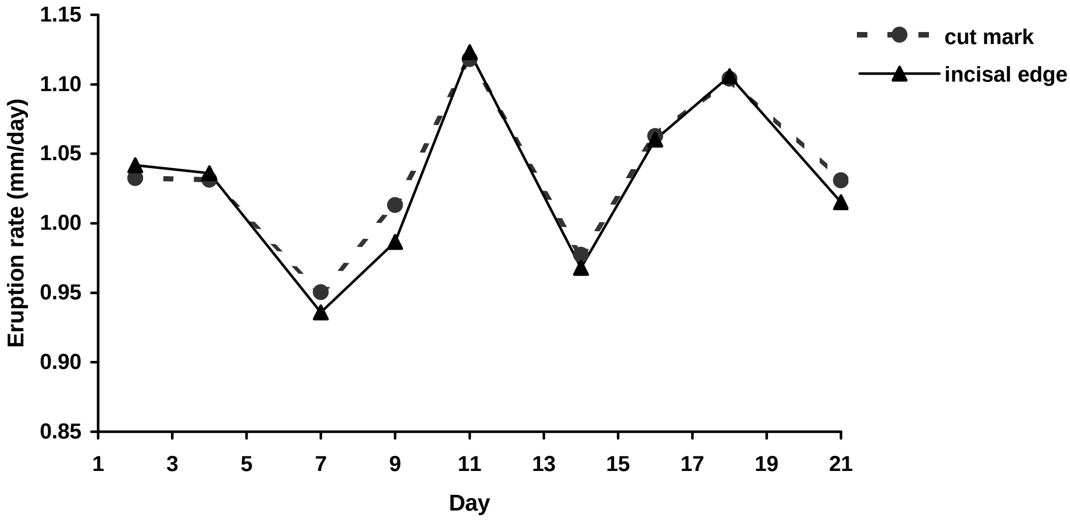


Figure 4. The unimpeded eruption rate measured by the image analysis method during the 3-week period with the incisal edge and the cut mark as reference points.

Reading	Method		p value
	optical (mm)	image analysis (mm)	
First	6.46±0.53	6.66±0.53	p=0.735
Second	6.46±0.52	6.67±0.53	p=0.994
Mean	6.46±0.53	6.66±0.52	p=0.819

Table 1. A comparison of the length (mm) of the left incisors measured by the optical and image analysis methods (n=272).

Day	Control group (n=12)		Experimental group (n=22)		
				left unimpeded incisor	
	right incisor	left incisor	right impeded incisor	cut mark as reference point	incisal edge as reference point
Day 2	*	*	*	*	p=0.0285
Day 4	p=0.008	*	*	*	*
Day 7	*	*	*	*	*
Day 9	*	*	*	*	*
Day 11	*	*	*	*	*
Day 14	*	*	*	*	*
Day 16	*	*	*	*	*
Day 18	*	*	*	*	*
Day 21	*	*	*	*	*

* = not significant (p>0.05)

Table 2. A comparison of the eruption rates obtained by the optical and image analysis methods.

Optical method		Image analysis method	
cut mark as reference point	**incisal edge as reference point**	**cut mark as reference point**	**incisal edge as reference point**
7 (3-day interval)	7 (3-day interval)	7 (3-day interval)	7 (3-day interval)
2 (2-day interval)	2 (3-day interval)	14 (3-day interval)	14 (3-day interval)
9 (2-day interval)	21 (2-day interval)	9 (2-day interval)	9 (2-day interval)
14 (3-day interval)	14 (3-day interval)	21 (3-day interval)	21(3-day interval)
21(3-day interval)	9 (2-day interval)	4 (2-day interval)	
		2 (2-day interval)	

Table 3. Results of Student-Newman-Keuls tests showing groups of 2-day and 3-day intervals with mean unimpeded eruption rate in homogeneous subsets. The groupings were obtained by the image analysis and optical methods using incisal edge or cut mark as reference points.

	Optical method		Image analysis method	
	number of rats	differences in the length of the two incisors (mm)	number of rats	differences in the length of the two incisors (mm)
Day 2	22	0.70	22	0.81
Day 4	22	0.63	22	0.73
Day 7	17(5)	0.21 *	17(5)	0.23 *
Day 9	21(1)	0.69	22	0.71
Day 11	22	0.55	22	0.65
Day 14	19(3)	0.28 *	19(3)	0.34 *
Day 16	22	0.65	22	0.75
Day 18	22	0.78	22	0.91
Day 21	22	0.44 *	22	0.49 *

Readings with * represent the lengths smaller than 0.5mm

Table 4. Differences in the length of the two incisors in the experimental group before shortening after 2-day and 3-day intervals. The figures in parenthesis denote the number of rats which had an unimpeded incisor longer than the adjacent impeded left incisor and these data were ignored in the final analysis.