

A Hypothesis: Autonomic Rhythms are Reflected in Growth Lines of Teeth in Humans and Extinct Archosaurs

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conserved throughout evolution. Details of the rhythms give clues to life styles in ancient civilizations and to the physiology of extinct archosaurs.

Abstract

A major determinant of tooth architecture is the arrangement of lines in dentin and in the enamel, following the contour of the surface. Since the original description of these lines in the 19th century they have been attributed to recurring events during tooth development. They have also attracted the attention of dental scientists and anthropologists; however, to date, studies of these structures have been largely theoretical and microscopic. We show here that the statistical properties of the spacing between the lines are similar in teeth from both ancient and modern humans and from extinct archosaurs-reptiles that lived tens or hundreds of millions of years ago- they also resemble heart rate variability of living humans. We propose that the deposition of these recurring structures is controlled by the autonomic nervous system (ANS). This control accounts for their regularity and recurrent nature and implies that the lines are an expression of a biologic rhythm which has been

Introduction

Sections of human enamel show lines along the contours of the crowns. These striae of Retzius (SR) (Retzius, 1837) recur at regular intervals. Lines in the dentin, the lines of Andresen (LA), also are regularly spaced (Hillson, 1996). The surface expressions of SR's in human teeth are the perikymata grooves (PG) (Moggi-Cecchi, 2001). Archosaur teeth have analogous dentin lines, whose surface expressions are the growth lines (GL) (Johnston, 1979) These lines are deposited at 8 to 9-day intervals, a long period rhythm (LPR) (Hillson, 1996). While it is likely that these recurring lines reflect biologic rhythms (Dean et al., 2001), their control has remained mysterious.

Animals show many other intrinsic rhythms, such as daily or monthly variations in hormonal concentrations, blood pressure and temperature. Underlying them are neuronal intracellular clocks that reflect changes in gene expression in the suprachiasmatic nucleus of the hypothalamus, the brain region regarded as the master time keeper. This region regulates the expression of genes encoding other clock components that are part of a network of clock neurons in other parts of the brain (Reick et al., 2001). The signals from these networks reach peripheral tissues via the ANS (Buijs et al., 2003).

Materials and Methods

Our goal was to show that the regularly spaced lines in teeth reflect a biologic rhythm governed by the ANS. Biologic rhythms are greatly affected by extraneous influences such as light, darkness and temperature. Because these influences on human biologic rhythms have changed over time, with the advent of artificial light or rigid insulated shelters, we sought to support our findings by comparing the biologic rhythms of ancient humans with their modern counterparts.

Using dissecting and confocal microscopy, we compared human LPRs by studying teeth from three different cultures, two ancient and one modern. We measured the spacing between SR in 100 μ m sections of 8 third molars from the San Pedro culture, which inhabited oases near present day San Pedro de Atacama, Chile from AD 400-900, and the spacing between PGs in 11 third molars from the Chiribaya culture near Ilo, Peru (AD 900-1350), a coastal civilization that left traces near the Atacama oases. We compared the SR and PG spacing in ancient teeth with measurements of SR in 21 modern third molar teeth from Albuquerque, NM USA (Fig. 1).

Our second goal was to support our contention that the recurring patterns in teeth reflect biologic rhythms by examining the growth lines of archosaur teeth from various epochs. Because of the importance to survival of such rhythms we predicted that they would be conserved throughout evolution.

We measured GL spacing in the teeth of 17 tyrannosaurid dinosaurs, a species that lived 70 million years ago (MA), 16 crocodylians (70 MA), 1 Brachiosaurus dinosaur (150 MA), 32 Allosaurus dinosaurs (159 MA), and 23 phytosaurs (225 MA). We applied confocal laser microscopy image analysis to the Brachiosaurus tooth and to sections from 5 modern and 5 ancient human teeth. We measured the others visually, using a dissecting microscope. We then analyzed the spacing, using frequency domain analysis, a statistical tool developed for the study of normal and pathological cardiovascular function and for the assessment of the chronobiology of the heart (Malliani et al., 1991).

Two main oscillatory components of heart rate cadences have been recognized by power spectrum analysis using this method: a low frequency (LF) component at about 0.1 s⁻¹ (Hz), and a high frequency (HF) component at about 0.25 s⁻¹ (Hz) (Malliani et al., 1991). The spectral index of HF reflects parasympathetic control of the heart, and the index of LF, sympathetic control. In normal and pathological conditions, the ratio LF/HF expresses the balance of sympathetic and vagal tone (Malliani et al., 1991). The spectral indices obtained by recording heart rate variability do not reflect quantitative neural signals. They only show the dynamic interaction between the neural control of the heart and its response (i.e. the sino-atrial node discharge). Applying the same technique to the human and archosaur tooth measures, we identified the presence of two similar frequency bands for normalized axes whose frequencies are expressed in cycles/deposition for teeth and cycles/beat for heart rate (Fig. 1). A slow frequency (SF),

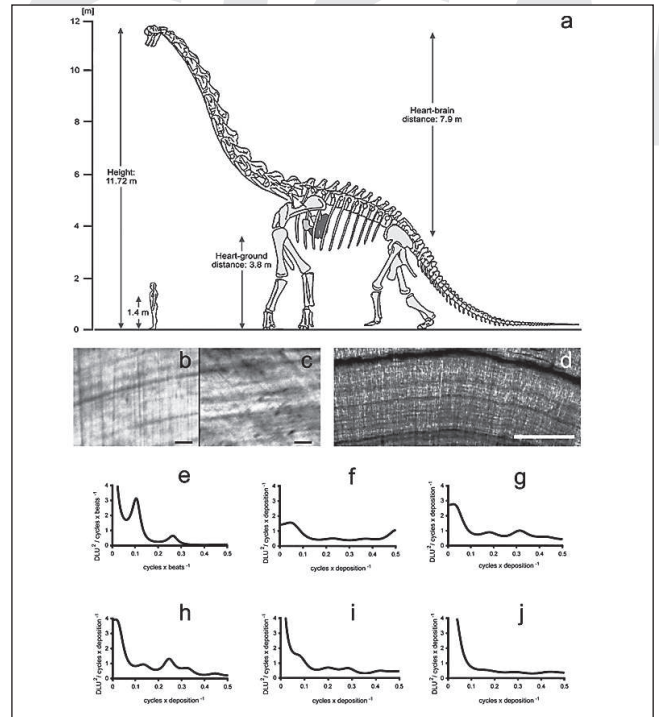


Fig. 1 - To keep animals' brains adequately supplied with blood, the heart speeds up or slows down under the control of the autonomic nervous system. Rhythmic variations in these responses can be studied by power spectral analysis of heart rate variability (Malliani et al., 1991). These rhythms also leave a record in teeth. a. Schematic representation of hydrostatic influences on blood supply to the brain of a human being and a Brachiosaurus dinosaur. Hydrostatic influences on brain-blood supply increase as the brain's distance from the heart increases (for humans 30-35 cm). A change in posture from supine to upright results in displacement of blood to dependent vessels, but the heart speeds up to compensate and keep blood pressure and flow to the brain from falling this is apparent from an increase in low frequencies in the power spectrum of heart rate variability (Malliani et al., 1991). The dinosaur weighed approximately 40 tons (heart brain distance 7.9 m) compared to an average body weight of 70 kg for humans. Comparisons of this dinosaur with a healthy man (in parentheses) show: Blood volume [l] 3660 (5.4); Heart weight [Kg] 386 (0.32); Heart rate [min.⁻¹] 14.6 (70); blood pressure [mmHg] 400 (120). Respiration rate [min.⁻¹] 3 (15); b. Section of human third molar from the San Pedro culture (AD 400-900). The spacing between the two darker gray lines, the striae of Retzius, arching from left to right, a long period rhythm (LPR), was measured in this dissecting microscope image. c. Section of modern human third molar. The spacing of the three darker gray lines arching from left to right, the striae of Retzius, LPR, was measured in this dissecting microscope image. d. Section of Brachiosaurus dinosaur tooth from near Tendaguru Lake, Tanzania (Late Jurassic, 150 million years ago). The spacing of the two dark lines arching across this laser microscope image, LPR, was measured by an image analyzer. e. Power spectral density (PSD) of heart rate variability (cycles x beats⁻¹) from a 37 year old healthy man. f. PSD of spacing measure variability (cycles x deposition⁻¹), LPR, obtained from the striae of Retzius from modern third molars. g. PSD of spacing measure variability obtained from San Pedro culture third molars (AD 400-900). Note that the PSD of this LPR is more pronounced than in f reflecting the greater impact of external entrainment on this biologic rhythm in ancient times. h. PSD of spacing measure variability, LPR, obtained from growth lines of tyrannosaurid dinosaurs from what is now New Mexico. This power spectrum closely resembles the LPR spectrum of modern human heart rate variability (see: e.) i. PSD of spacing measure variability obtained from growth lines of the Brachiosaurus dinosaur, from Tanzania. This animal lived at a latitude of approximately 15° south where climatic conditions at that time did not differ significantly from those that the tyrannosaurids experienced, its PSD was not significantly different from other archosaurs and closely resembled PSD of modern human teeth (see: f) j. PSD of spacing measure variability obtained from growth lines of crocodylians. Note the significantly lower PSD of this aquatic species. Scale bars: b and c 100 μ m, d 200 μ m, LPR long period rhythm, e-j normalized autoregressive power spectra, DLU dimensionless units.

from 0.04 to 0.14 cycles/deposition and a fast frequency (FF), from 0.15 to 0.4 cycles/deposition in tooth measure spectra and a low frequency (LF), from 0.04 to 0.14 cycles/beat and a high frequency (HF), from 0.15 to 0.4 cycle/beat in human heart rate variability spectra. We computed the power in the above mentioned bands, standard errors and SF/FF and LF/HF ratios. The power of the bands, and ratios were compared by ANOVA and t-tests, and the results were checked by non-parametric analyses.

We measured the spacing between striae of Retzius in 100 μ m ground sections of human third molars. We tilted a fiber optic light source to provide a slanted beam to the surface of the teeth to visualize the perikymata grooves and measured the spacing between the grooves on the surface of third molars. We applied the same technique to measure spacing of growth lines on the surface of extinct archosaur teeth. For these measures we used a dissecting microscope and micrometer.

To check our measurements we utilized a confocal laser image analyzer to measure the spacing of the lines of Andresen in section of dentin of Brachiosaurus teeth and the spacing between striae of Retzius in sections of 5 ancient and 5 modern human teeth previously assessed by dissecting microscopy.

Because of insufficient numbers of measurements available from single teeth (except for those obtained from the Brachiosaurus) we pooled the measures from each taxon and considered the pools as individuals.

We substituted the spacing measures from the teeth for the heart beat interval measures used for the computation of power spectra of human heart rate variability and applied an accepted method to construct power spectra based on the fluctuations of these spacing measures (Malliani et al., 1991). We compared the spectra obtained from human and terrestrial archosaur teeth with 48 normal human heart rate variability spectra.

Statistics.

We compared the power spectra using unit-less values such as the low frequency/high frequency ratios and area under standardized spectral density curves. We used Priestley's method (Priestley, 1981) to obtain standard errors (SE) for the average of spectral densities. These SE represented 2 sources of variability: between individual teeth used in the pool of a taxon and the SE inherent in the time series of each individual tooth. We also used t-tests to compare the unit-less measures of groups. The t-tests were verified by non-parametric analysis (Wilkoxon).

Results

After normalization using parametric autoregressive spectral analysis all tooth LPR spectra were similar to the spectrum obtained from contemporaneous human heart rate (Fig. 1). Using non-parametric spectral analysis ancient and modern human tooth spectra did not differ statistically. The extinct aquatic Crocodylians and Phytosauria showed

lower SF/FF ratio ($p=0.001$) and lower FF ($p=0.02$) compared to terrestrial archosaurs (see Fig. 1 supplemental files). The length measure spectra obtained by confocal laser image analysis from the Brachiosaurus were not statistically different from those obtained by dissecting microscopy, but resembled modern rather than ancient human tooth spectra. Comparison of SF/FF ratios of spectra of ancient and modern human teeth and of terrestrial archosaurs with the LF/HF ratios of 48 normal human heart rate variability spectra showed no significant differences. There was no effect of antiquity or latitude of residence on human or archosaur spectra.

Discussion

Our successful application of the method for studying heart rate variability raises the possibility that the ANS also controls biologic rhythms in teeth. The ANS, through its control of cardiac activity and blood vessel diameter, modulates the amount of blood flowing to all tissues, including the teeth. The spacing measures of the recurring lines in human and archosaur teeth analyzed here reflect the variability of the rhythms of tooth growth. These recurring lines are attributed to a slowing of tissue deposition by ameloblasts, the cells that form enamel or odontoblasts, the cells that form dentin (Hillson, 1996). Thus, recurring falls in blood flow with consequent decrease in function of the relevant cells are likely an expression of ANS control of these biologic rhythms. Teeth, therefore, preserve the remains of biologic rhythms during the first 11 years of life (the time for full enamel development) in third molars and over the 2 year interval before the shedding of teeth in archosaur dentin. Details of these rhythms provide clues to physiology and lifestyle. The aquatic habit of crocodylians and phytosaurs places less demands on the ANS in counteracting gravity to maintain normal blood flow because of the animals' horizontal posture. This was evident from the lower SF/FF ratio and lower FF content of the spectra of these taxa. Enamel and skin, both derived from the ectoderm, grow predominantly during rest spans in darkness (Halberg et al., 1959). Human teeth from the ancient civilizations developed at a time when no artificial light influenced their growth. Furthermore, children in ancient times experienced a greater range of temperature and other climatic conditions than they do now, living in heated and air-conditioned houses. Such external effects on biologic rhythms during the development of modern teeth would have been much reduced. These and many other factors may account for the greater variety of spectral waves that we found in ancient compared to modern teeth. Similar considerations might also explain the likeness between modern human tooth spectra and those obtained from Brachiosaurus. This animal was somewhat insulated from external influences on its biological rhythms by its massive weight, just as modern humans are sheltered from the environment by our technology.

Further study may reveal other expressions of these biologic rhythms.

The otoliths of zebrafish are a biomineralized tissue, like teeth, and they have a layered structure similar to human and archosaur growth lines in teeth. Otolith structure is dependent on a recently discovered protein, "Starmaker" (Söllner et al., 2003). Although circadian or LPR variations in Starmaker expression have not, as yet, been assessed, these biologic rhythms are likely to be found because Starmaker is also expressed in the pineal (Söllner et al., 2003), an organ involved in many biologic rhythms. The recent finding of families with hearing loss and dental deformities hints at a genetic link between ear and tooth structure, and the Starmaker protein shares sequence homology with a human protein called dentin sialophosphoprotein (DSPP), involved in the biomineralization of teeth (Xiao et al., 2001). Taken together these results

suggest that a further extension of the brain's networks controlling biological rhythms in human and archosaur teeth might be rhythmic changes in the expression of Starmaker and other proteins.

We provide circumstantial evidence here for ANS control of biologic rhythms in human and extinct archosaur teeth that remained unchanged for epochs. These rhythms resemble power spectra of contemporaneous human heart rate variability. However, previously published data on human biologic rhythms, are insufficient for a rigorous test of our model. Furthermore, the applicability of this model to extinct archosaurs should be regarded as unproven until checked by further studies.

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Literature Cited

- Buijs RM, van Eden CG, Goncharuk VD, Kalsbeek A. 2003. Circadian and seasonal rhythms. The biological clock tunes the organs of the body: timing by hormones and the autonomic nervous system. *Journal of Endocrinology* 177: 17-26.
- Dean C, Leakey MG, Reid D, Schrenk F, Schwartz GT, Stringer C, Walker A. 2001. Growth processes in teeth distinguish modern humans from *Homo erectus* and earlier hominins. *Nature* 414: 628-631.
- Halberg F, Halberg E, Barnum CP, Bittner J. 1959. Photoperiodism and related phenomena in plants and animals. In: # 55 American Association for the Advancement of Science, Washington D p. 803-878.
- Hillson S. 1996. *Dental Anthropology*. Cambridge: Cambridge Univ Press. p. 373.
- Johnston PA. 1979. Growth rings in dinosaur teeth. *Nature* 278: 635-636.
- Malliani A, Pagani M, Lombardi F, Cerutti S. 1991. Cardiovascular neural regulation explored in the frequency domain. *Circulation* 84: 482-492.
- Moggi-Cecchi, J. 2001. Question of growth. *Nature* 414: 595-597
- Priestley MB. 1981. *Spectral Analysis and Time Series*, Volume 1, London: Academic Press p. 426.
- Reick M, Garcia JA, Dudley C, McKnight SL. 2001. NPAS2: An analog of clock operative in the mammalian forebrain. *Science* 293: 506-509.
- Retzius A. 1837. Bemerkungen über den inneren Bau der Zähne, mit besonderer Rücksicht auf dem in Zahnknochen vorkommenden Röhrenbau. (Müllers) *Archiv Anat Physiol Year* 1837, 486-566.
- Söllner C, Burghammer M, Busch-Nentwich E, Berger J, Schwarz H, Riekel C, Nicolson T. 2003. Control of crystal size and lattice formation by Starmaker in otolith biomineralization. *Science* 302: 282-286.
- Xiao S, Yu C, Chou X, Yuan W, Wang Y, Bu L, Fu G, Qian M, Yang J, Shi Y, Hu L, Han B, Wang Z, Huang W, Liu J, Chen Z, Zhao G, Kong X. 2001. Dentinogenesis imperfecta I with or without progressive hearing loss is associated with distinct mutations in DSPP. *Nat Genet* 27: 201-204.

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