Two-dimensional craniometry: morphometry and cephalometry

Craneometría bidimensional: morfometría y cefalometría

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Article History
Received: 16 11 20
Accepted: 21 04 21
Published: 27 10 21

ABSTRACT

Over the last twenty years, bone research has raised interest; the function, shape and alterations of bone tissue have been analyzed and reported. Craniometry is a method developed as a tool for the precise measurement of the skull and facial structures and it has been used not only for humans, but for a variety of mammals. Morphometry is used to study and quantitatively compare the shape variation of biological objects, organs and organisms. Cephalometry uses two-dimensional measurements of the head and face involving a radiographic analysis to provide data on soft tissue, dental and skeletal relationships. Papers regarding bone research are difficult to access and usually, morphometry and cephalometry are reported separately. The object of this article is to report a two-dimensional craniometry performed in rats, using both techniques. This specific craniometry is a functional, well-established method which involves the entire anatomical aspects of the skull.

Key Words: Bone and bones; cephalometry; mandible; rat; skull.

RESUMEN

En los últimos veinte años, la investigación ósea ha ganado interés; la función, forma y alteraciones de este tejido están siendo analizadas y reportadas. La craneometría es un método desarrollado para la medición precisa del cráneo y las estructuras faciales; no solo ha sido usada en humanos, sino, en gran variedad de mamíferos. La morfometría estudia y comparar cuantitativamente el cambio en la forma de objetos biológicos, órganos y organismos. La cefalometría utiliza medidas bidimensionales de la cabeza y la cara, involucrando análisis radiológico, para brindar datos sobre tejidos blandos, dientes y la relación esquelética. Estudios acerca del tejido óseo, son difíciles de conseguir y usualmente, reportan craneometría y morfometría, separadas. El objetivo de este artículo es reportar una craneometría bidimensional realizada en ratas, usando morfometría y cefalometría. Esta craneometría específica, es un método funcional y bien establecido, que involucra la anatomía del cráneo en su totalidad.

Palabras clave: hueso y huesos; crecimiento y desarrollo; mandibula; rata; cráneo.

DOI 10.17081/innosa.136

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How to cite this article: Botero-González D, Ortiz M, Herrera-Rubio AM. Two-dimensional craniometry: morphometry and cephalometry. Ciencia e Innovación en Salud. 2021. e136: 278-285 DOI 10.17081/innosa.136

I. INTRODUCTION

According to the National Research Council (US) and Institute of Medicine (US) Committee on the Use of Laboratory Animals in Biomedical and Behavioral Research, animal experimentation is the basic component of biomedical research. Its importance lies in the information it provides to understand diseases. In bone research, craniometry can offer qualitative and quantitative information about the size, the shape and the development of bone tissue. Gentile in 1952 reported that using external characteristics and cranial measurements often partially bases the descriptions of new forms on variations in measurements of different samples (1). The skull can be measured in a two-dimensional way, by either of these techniques: morphometry and cephalometry. Sarnat in 1997 stated that a combination of both techniques will provide further information, and additionally, it could be more accurate than using a single technique (2).

Morphometry contributes to a better understanding of bone biology and its disorders. It is based on analysis of distances, such as lengths, heights and widths. And has provided the methodological basis for non-geographic and geographic variation study in phenotypic morphological traits (3).

Cephalometry is used to research the growth and the development of the craniofacial structures. And different authors had shown that a longitudinal cephalometric method is reliable for describing skull morphology and growth in the rat (4).

It is very difficult to find in the academic literature a craniometry specifically developed to use in rats. Each author describes his or her own method and adjusts it to the specific area of interest. Besides, the morphometric and cephalometric techniques are frequently used separately. To our knowledge, there is no established protocol that allows a full craniometry of rats. Therefore, it is the purpose of this paper to describe a method for a two-dimensional craniometry used for rat skulls that includes both morphometric and cephalometric measurements.

II. MATERIALS AND METHODS

This research had the endorsement of the “Comité Institucional de Revisión de Ética con Animales en Experimentación, Universidad del Valle” internal code 015-019.

Both morphometry and cephalometry were used to establish a protocol for a two-step craniometry in Wistar rat dry skull, to evaluate the entire craniofacial complex.

MORPHOMETRY

The sample was brought under the stereomicroscope (MEIJI Techno, RZT Stand) (5) attached to a camera (Infinity, reference 3), where lateral, dorsal and ventral images were taken, using an image software (Infinity 5.0.3.) (6,7). Calibrated stereomicroscope was set to 7.5 x and bi/photo. Upper and lower light were placed. Distance from the platform to the cone, was 20 centimeters. Parameters were set for the software, such as: saturation 1, matrix 0, brightness 8 and contrast 20. A scale in millimeters was always placed next to the skull sample as a reference.

Morphometric measurement was made on the digital two-dimensional photographs, using the image tool software (ImageJ, available from NIH site). The pixel scale was set to 57 per millimeter. Morphometry was based on morphometric landmarks and distances Richtsmeier et al., 2000, Fernandes et al., 2008, and Yang et al., 2011(5-7). **Figure 1 and table 1.**

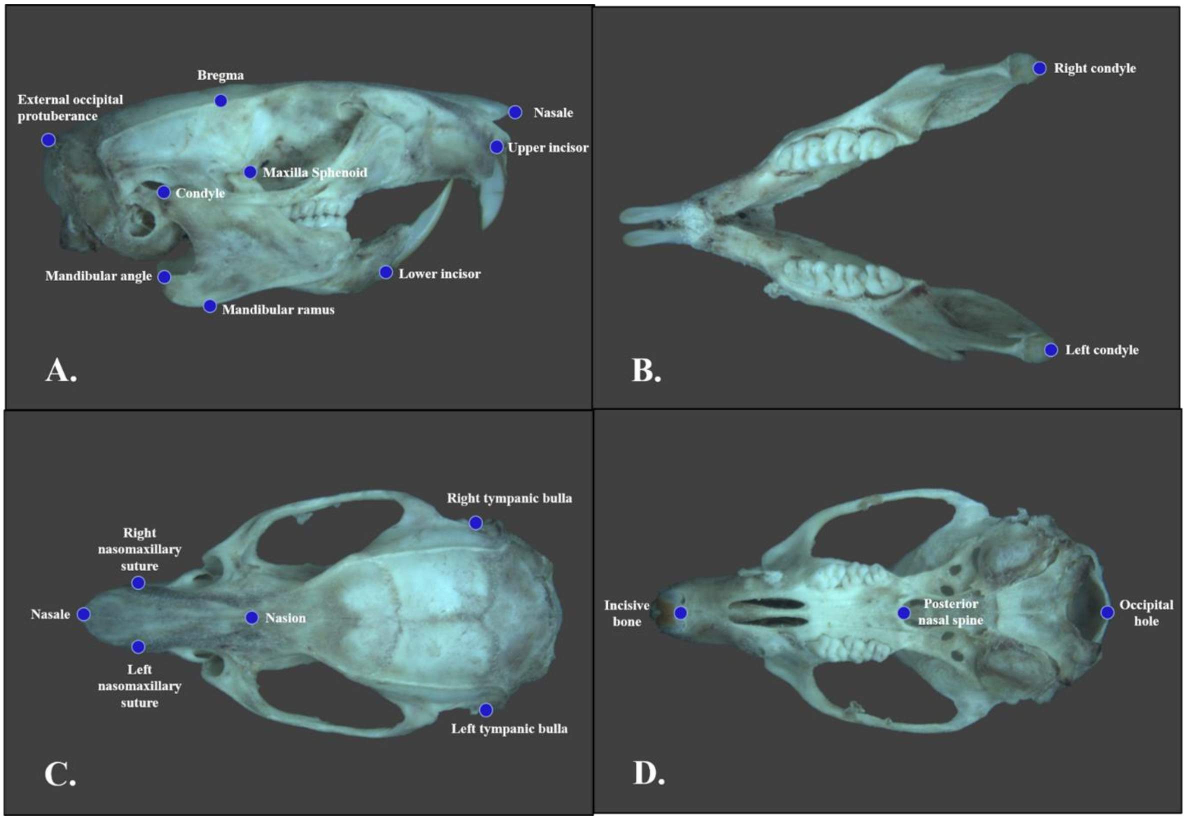

Figure 1. Skull morphometrics landmarks

A. ***Lateral view:*** Nasale: Inferior-most point of intersection of the nasal bones; External occipital protuberance: Intersection of interparietal and occipital bones at the midline; Bregma: Intersection of frontal and parietal bones; Mandibular ramus: The inferior-most point on border of mandibular ramus; Upper incisor: Center of alveolar ridge over maxillary incisor; Maxillary sphenoid: Intersection of maxilla and sphenoid on interior alveolar ridge; Lower incisor: Inferior-most point on incisor alveolar rim; Mandibular angle: Mandibular angle; Condyle: Posterior-most point on mandibular condyle. **B.** ***Dorsal view of the mandible:*** Right condyle: Posterior-most point on mandibular right condyle; Left condyle: Posterior-most point on mandibular left condyle. **C*****. Dorsal view without the mandible:*** Nasale: Inferior-most point of intersection of the nasal bones; Nasion: Point of intersection of the nasal bones with the frontal bones; Right nasomaxillary suture: Right margin of the nasomaxillary suture; Left nasomaxillary suture: Left margin of the nasomaxillary suture; Right tympanic bulla: Anteromedial border of the right tympanic bulla; Left tympanic bulla: left tympanic bulla. **D.** ***Ventral view without the mandible:*** Incisive bone: he alveolar margin of the incisive bone in the median plane; Posterior nasal spine: Posterior-most point of intersection of the maxillary bones on the hard palate; Occipital hole: More dorsal aspect of the occipital hole.

Table 1. Morphometric distances

Distance	***Description***
Nasale-External occipital protuberance	Maximum length of the neurocranium
Bregma-Mandibular ramus	Skull height
Upper incisor-Maxilla Sphenoid	Maxillary length 1
Lower incisor-Mandibular angle	Mandibular corpus length
Lower incisor-Condyle	Mandibular length
Mandibular angle-Condyle	Mandibular ramus length
Right condyle-Left condyle	Mandibular width
Nasale-Nasion	Nasal length
Right nasomaxillary suture-Left nasomaxillary suture	Nasal width
Right tympanic bulla-Left tympanic bulla	Skull width
Occipital bone-Incisive bone	Skull base length
Incisive bone-Posterior nasal spine	Maxillary length 2

CEPHALOMETRY

Due to the need to stabilize the skull sample, it was placed on a parallelometer (DAC) where it was attained that both right and left mandibular corpus and the maxilla where perpendicular to the surface. Generic dental modelling wax was used to establish the position.

A standard human dental X-ray machine (Gendex, Gx-770) was used at 70 kV and 7 mA. The length of exposure was 7 pulses. The distal part of the cone to the film was set to a distance of 3.5 centimeters. The film was a digital periapical film for human teeth. The film was developed at a digital intraoral imaging plate system (DIGORA, Optime DXR-50 001). The digital two-dimensional radiography was a 1:1 scale. Radiography was printed and copied in a standard cephalometric tracing paper.

The cephalometric analysis was made manually, and it contained both linear and angular measurements and was adapted from Engström et al., 1982 and Abbassy, et al, 2008 (8-9). **Figure 2 and table 2.**

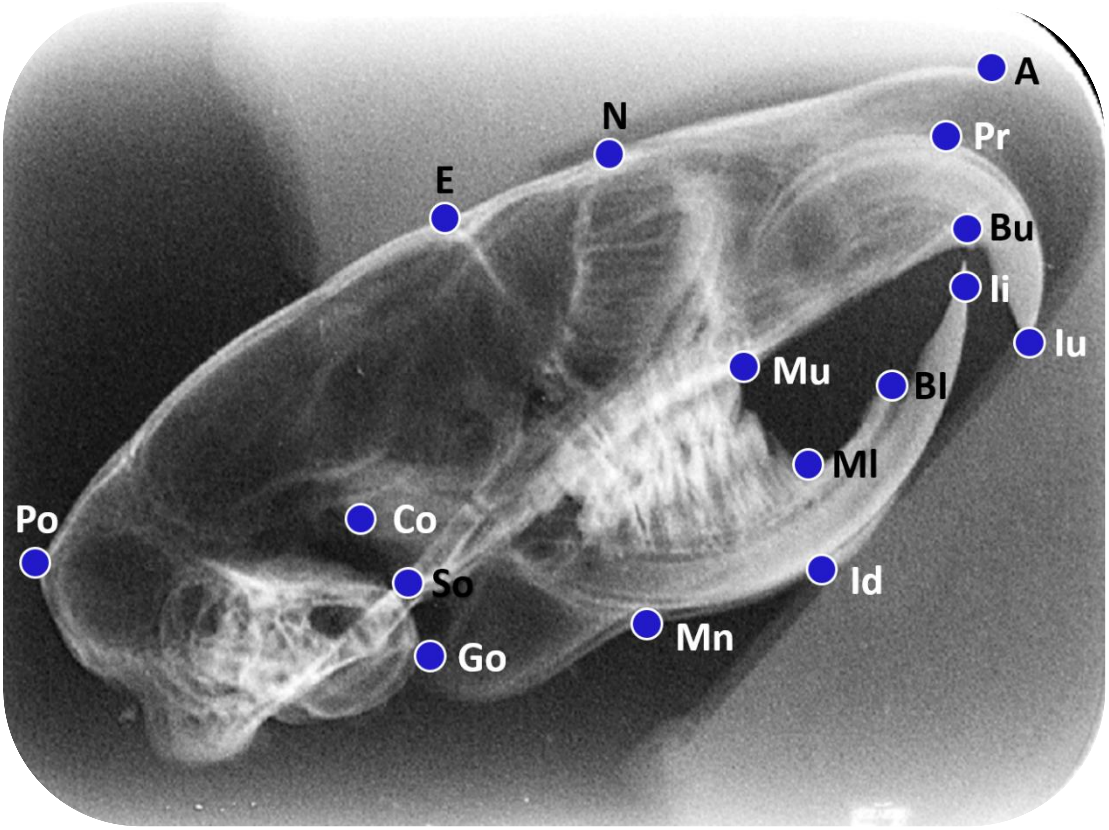

Figure 2. Cephalometric points.
Po: The most posterior point on the cranial vault; N: A point on the nasofrontal suture; A: The most anterior point on the nasal bone; E: The intersection between the frontal bone and the most superior-anterior point of the posterior limit of the ethmoid bone; So: The intersection between the posterior border of the basisphenoid and the tympanic bulla; Pr: The most inferior and anterior point on the alveolar process of the premaxilla; Bu: A point on the premaxilla between jaw bone and the lingual surface of the upper lingual incisors; Iu: The most prominent point between the incisal edges of the upper incisors; Mu: A point on the intersection between the maxillary bone and the mesial surface of the upper first premolar; Ii: The most prominent point between the incisal edges of the lower incisors; Id: The most inferior and anterior point on the alveolar process of the mandible; Mn: A point in the deepest part of the antegonial notch curvature; Ml: A point on the intersection between the mandibular alveolar bone and the mesial surface on the first premolar; Bl: A point on the intersection between the lingual surface of the lower incisors and the most anterior part of the lingual alveolar bone; Co: The most posterior and superior point on the mandibular condyle; Go: The most posterior point on the mandibular ramus.

III. RESULTS

A two-dimensional craniometry protocol was established, using both morphometry and cephalometry. This specific craniometry is a functional, well-established method which involves the entire anatomical aspects of the rat skull.

Morphometry was established by the use of 20 anatomical landmarks and 12 morphometric distances. Cephalometry was set up by the use of 16 points, 17 distances and 16 angles. **We invite you to consult the supplementary material.**

Table 2. Cephalometric distances and angles.

Distance or Angle	***Description***
Po-A	Total skull length
Po-E	Neurocranial length
E-A	Anterior skull base length
So-E	Posterior skull base length
N-A	Nasal bone length
E-Mu	Viscerocranial height
E-Pr or E-Bu	Viscerocranial length
Mu-Bu or Mu-Pr	Palate length
E-Iu or Iu-Pr	Erupting upper incisor length
Mn-Id	Mandibular corpus length
Ii-Id	Buccal lower incisor length
Ml-Ii	Lingual lower incisor length
Go-Mn	Posterior mandibular corpus length
Co-Bl	Total mandibular length
Po-E/So-E	Neurocranial length to cranial base length
E-A/So-E	Anterior skull base length to posterior skull base length
A-N/So-E	Nasal bone length to posterior skull base length
A-N/Po-E	Nasal bone length to neurocranial length
E-Pr/So-E or E-Bu/So-E	Viscerocranial length to posterior skull base length
Mu-Bu/Po-E or Mu-Pr/Po-E	Palate length to neurocranial length
Mu-Bu/So-E	Palate length to posterior skull base length
E-Iu/Po-E	Erupting upper incisor length to neurocranial length
E-Iu/So-E	Erupting upper incisor length to posterior skull base length
Mu-Pr/Iu-Pr or Mu-Bu/Iu-Pr	Palate length to erupting upper incisor length
Mn-Id/So-E	Mandibular corpus length to cranial base length
Ml-Ii/Ii-Id	Buccal lower incisor length to lingual lower incisor length
Co-Bl/So-E	Total mandibular length to cranial base length

This protocol can be reproduced in any laboratory that has the equipment used here or even with less/more advanced technology equipment. It will benefit anyone interested in bone research, development of this hard tissue, or the observation of morphometric changes in the skull.

IV. DISCUSSION

The vertebrate skull is an intricately designed, evolutionarily ancient (5) that has proved to be a rich source of studies. It particularly has raised interest because of the different types of bone growth, the increase in size and different cavities, and the growth and eruption of teeth (2).

Anatomically, the skull is defined as the skeleton of the head, including the facial bones and the bones enclosing the brain.

Craniometry can be performed on either the living or dried subject specimens. When performed on the living, the measurement instruments must be placed on the soft tissues overlying the bony landmarks, thereby precluding precise accuracy of measurements (2).

Morphometric landmarks on the skull offer the advantage of maintaining an anatomical position and can be consistently and reliably located, with a measurable degree of accuracy. The linear measurement based on those landmarks can give us the knowledge of forms (10).

In the nineties, Lele and Richtsmeier described a method to statistically define a biological form using landmarks on dried skulls for humans and monkeys called the Euclidean Distance Matrix Analysis (EDMA) (10). Later on, Richtsmeier et al., used the same EDMA to evaluate dried mice skulls, including linear distances (5). In recent studies, Fernandes et al., outlined morphometric parameters to evaluate the width, length, and height of the rat skull (6). Yang et al, used mice to establish 44 skull landmarks and seven linear distances, and also a strong method for mandibular morphometry (7).

In regards to the cephalometry technique, the value of the X-ray method is twofold: it allows for the analysis of tissues, both qualitatively and quantitatively. Authors like Spence, define that a lateral X-ray of a rat skull reveals structural details such as sutures, sinuses, diploe and the important bones of the skull and the complete masticatory system (11).

In the early sixties, Asling and Frank developed a rat cephalometry technique based on seven linear distance measurements on dorsoventral and lateral view (12). Later, Engström et al, described a most ample technique of rat cephalometry to obtain an individual cephalometric description of normal growth and form, during postnatal growth. It was adapted from analyses described for guinea-pigs and rabbits, and contained linear and angular measurements (8). In more recent studies, Abbassy et al., established a broader cephalometry for the measurement of the mandible (9).

V. CONCLUSION

Rats have bones like any other vertebrate animals and the position of the skull only changes in relation to the constant growth of their teeth. The protocol described is a practical, authentic method which involves the entire anatomical aspects of the skull.

Craniometry for this murine comprise anatomical landmarks, morphometric distances, cephalometric distances and angles. This technique is a two-dimensional craniometry, using both morphometry and cephalometry. It can be applied to any mammal and can be performed in any laboratory. This research approach contributes to enhance bone biology and craniofacial research.

Funding: Vicerrectoría de Investigaciones [CI 1915] Universidad del Valle, Cali, Colombia.

Acknowledgements: Special thanks to Arturo Porras and Gloria González for critical reviewing the article.

Conflicts of interest: The authors have no conflict of interest to declare.

Authors contribution: DBG: Conceptualization, data curation, funding acquisition, investigation, methodology, visualization, and writing-original draft. MO: Conceptualization, funding acquisition, validation, and writing-review &editing. AMHR: Resources, validation, and writing-review &editing.

Article with supplementary material available

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