Original article

Effects of altered posture on the craniofacial growth in rats : A longitudinal cephalometric analysis

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Key words : posture, head extension, craniofacial growth, soft tissue stretching, functional matrix

Abstract : To clarify the long term effect of altered posture on craniofacial growth, eighty Wistar male rats of 25 days old were divided into two experimental (E1, E2), one control (C) and one normal (N) groups and raised in different postures for 60 days. Head extension was induced in E1 and E2 by keeping the rats in restrictive cylindrical cages, either horizontally for E1, or tilted upwards at 45° for E2. For a comparable overall growth, group C were kept in elliptical cages in which animal could be allowed much free than that both E1 and E2. Body and head radiographs were taken at 25, 55 and 85 days old to evaluate posture and any alterations in craniofacial growth. Craniocervical muscles were dissected out and weighed at 85 days old and the proportional muscle weight to body weight was calculated to analyze whether different posture had caused changes in muscle mass. Head extension was confirmed in both E1 and E2. Compared to control animals, E2 showed generally larger rate of muscle to body weight. Growth retardation was recognized in both craniofacial size primarily in E1, shown by smaller craniofaical length and height, and craniofacial rotation primarily in E2, shown by a downward rotation of the upper viscerocranium and the mandible. It was suggested that the stretching of the caraniocervical muscles induced by head extension might generate a strain force which restrained forward development of craniofacial complex and altered its functional matrix activity consequently to influence the craniofacial form and growth pattern.

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ラット顔面頭蓋の成長に及ぼす姿勢の影響
―頭部X線規格写真による縦断的分析―

抄録：長期の姿勢の変化が顔面頭蓋の発育に及ぼす影響を明らかにするために，80匹のウイスター系雄ラットを25日齢で実験群2群（E1, E2）と対照群（C），正常群(N)各20匹に分け，それぞれ異なる姿勢で60日間飼育した．E1群とE2群は頭部を伸展させるために狭い円柱状のかごに入れ，E1群のかごは水平に，E2のかごは45°上向きに傾けて設置した．C群は実験群より高に余裕のある楕円柱状のかごに入れて頭部を伸展しない姿勢で飼育した．N群は通常のケージの中で自由な姿勢で飼育した．姿勢と顔面頭蓋の成長変化を評価するため，25日齢，55日齢，85日齢で全身のX線写真と頭部X線規格写真を撮影した．85日齢で，体重に対する頭頸部の筋の重量比を求めた．姿勢について，E1, E2両群とも頭部が伸展していた．E2群では頭頸部筋の重量比が有意に大きかった．C群に比べて実験群では顔面頭蓋の成長低下が認められた．E1群では顔面頭蓋の前後径と高径が有意に小さく，切歯は舌側傾斜していた．E2群では顔面頭蓋が後下方へ回転していた．頭部の伸展は顔面頭蓋前方部への張力を生じ，機能的な構成要素の活動を変化させて，顔面頭蓋の形態と成長パターンに影響を及ぼすことが示唆された．

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Introduction

Posture is known to be determined by both the static morphology (such as bones, ligaments and muscles) and the dynamic function (such as strain or relaxation)[1]. Orthopedic and orthodontic studies have demonstrated the close relationship between the spine morphology and body posture[2] as well as between the cervical columns and head posture[3~6]. Previous orthodontic researches have indicated that head posture is closely correlated with craniofacial morphology. An association was found between head posture relative to the cervical column and craniofacial morphology[7] and between head posture and vertical or horizontal jaw relationship[8~10]. As a consequence of these findings it was suggested that the stretching of craniocervical soft tissues was one of the controlling factors in craniofacial morphogenesis[11].

On the other hand, reflexes maintain head posture according to physiological requirements, responding to variety of neural afferent stimuli. The head balance is maintained by the vestibular equilibrium mechanism of the otic capsule, in which the utricle chiefly concerned with static position of the head and the semicircular canals are responsive to kinetic movement of the head, chiefly rotation[12]. Anthropologists found that head balance was associated with the different craniofacial morphologic patterns[13]. Bipedal animal experiments have reported that altered body pasture led to the rotation of otic capsules and alteration of the spine curvature and the craniofacial morphology[12,14].

Electromyographic studies have demonstrated that head extension was correlated with a decreased tonic activity in the posterior cervical muscles and an increased activity in the lateral cervical, the supra-and infrahyoid muscles[15]. However, these muscular findings can not be directly applied to long term alterations of head posture, as the muscles may exhibit adaptive changes in relation to their tension and/or activity. According to the functional matrix hypothesis of Moss[16], craniofacial growth in size, shape and spatial position is influenced by the function of the soft tissues responding to their temporal demands which then mould the skeletal structures into their definitive forms. However, studies on body orientation is done only in bipedal animal experiments and the way how does body orientation affect craniofacial morphology remains unclear.

The present study was designed to clarify the long term effects of altered both head posture and body orientation on the craniofacial growth in rats and to explore possible factors involved in any biological mechanism for those changes.

Materials and methods

Animal posture and raising conditions

Eighty Wistar male rats, 25 days old, obtained from kyudo Co., Kumamoto, Japan, were divided into two experimental (E1, E2), one control (C) and one normal (N) group, with 20 animals in each group, and allowed to grow in different postures for 60 days. The method employed to change the animal's posture was illustrated in Fig. 1. To induce head extension, E1 and E2 were kept in restrictive cylindrical cages, made with overlapping wire-mesh which could expand in diameter to allow for body growth and orientated horizontally for E1 whilst tilted upwards at 45° for E2 to alter the animal's body orientation. As two experimental groups were kept in restrictive conditions, their general growth might be inhibited, group C were also kept in restrictive conditions in the horizontal orientation. Their cages were elliptical so that rats could move more freely than those of experimental groups but should not extend their heads. Group N were totally free in normal breading cages.

The cages of E1, E2 and C were put into normal breeding cages as used for group N to make animal care more convenient. Every morning, cages were cleaned and food and water were provided from the anterior top of cages for E1 and C whilst from the superior top for E2 as depicted in Fig 1. Illumination was provided from a fluorescent lamp which was lit from 7 a. m. to 7 p. m. in a room maintained at 24° C and 55% humidity.

Record of body and muscle weight

Body weight was recorded once a week during the whole experimental period. At the end of experiment (85 days old), the following cervical muscles of the right side were dissected from their origins to insertions. They were 1) masticatory : masseter (MA) and temporalis (TM), 2) suprahyoid : anterior digastricus (DA) and mylohyoideus (MH), 3) infrahyoid : sternohyoideus (SH) and omohyoideus (OH), 4) lateral cervical : sternomastoideus (SM), 5) prevertebral : longus capitis (LC) and 6) pos-

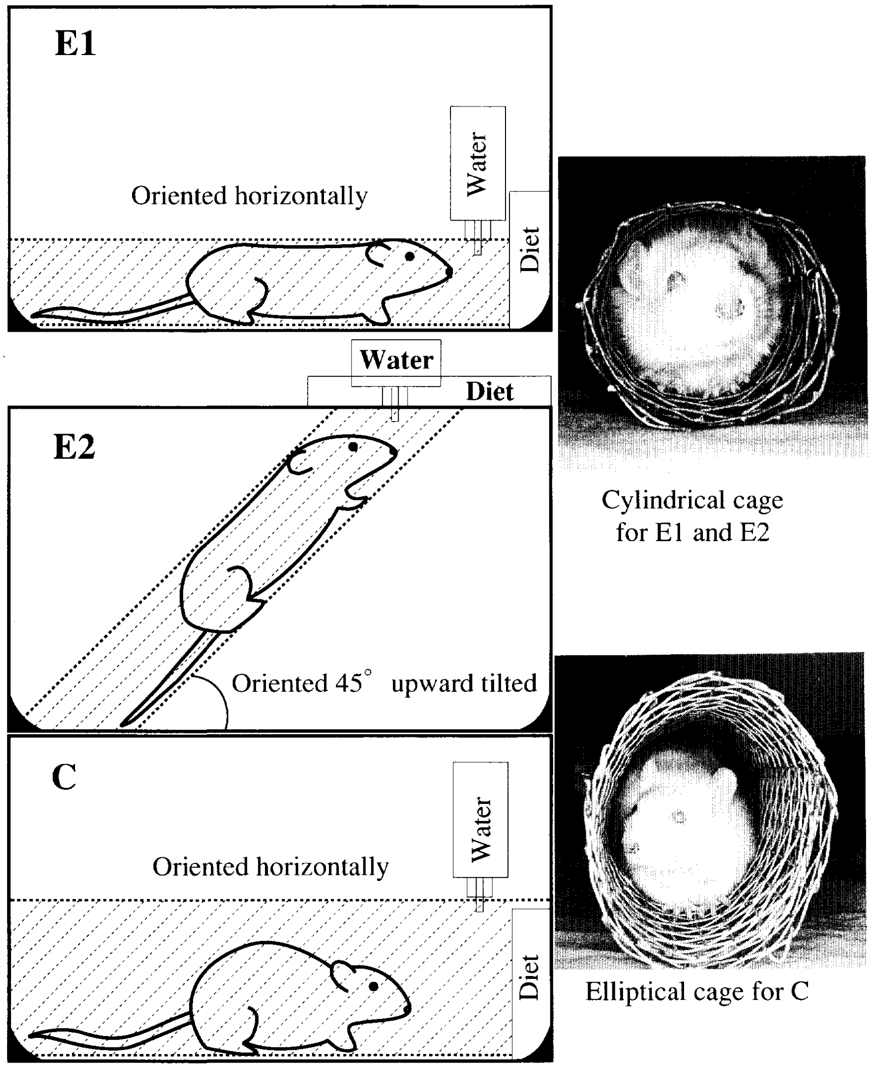


Fig 1 Posture and raining conditions for rats
Groups E1 and E2 animals were raised in restrictive cylindrical cages to induce head extension and oriented either horizontally for E1, or tilted upwards at 45° for E2. Group C were raised in elliptical cages much free than E1 and E2 but without head extension in horizontal orientation. To facilitate maintenance of the animals, the cages of E1, E2 and C were placed within normal breading cages and food and water were provided from the anterior top for E1 and C, whilst from above for E2

terior cervical : acromiotrapezius (AT).

Using an electronic balance (ER-120A, A&D Co., Japan), wet weight of each muscle was weighed immediately after sacrifice. Muscle weight was divided by body weight to get the proportional weight of each muscle.

Radiographic registrations

Body and cephalometric X-rays of the rats were taken with a standard dental X-ray machine (D-60-S, Electric RPG. Co. Japan) at 25, 55 and 85 days old. Body radiographs were taken in the natural rest position using an accelerating voltage of 95 KV and exposures of 2 sec. Without anesthesia, rats were kept in a plastic box of which inner size could be adjustable for their body sizes like their cages. All rats were taken repeatedly with two-hour intervals between exposures. The distance from the focus of the X-ray beam to the median plane of the rat body was 52 cm and there was 3 cm from the median plane of the rat body to the film.

The lateral (LA) and the axial (AX) cephalograms were taken using an accelerating vltage of 95 KV and exposures of 1.5 sec, under general anesthesia using intraperitoneal injections of pentobarbital sodium (25 mg/kg body weight). The rat heads

Table 1 Reference points and lines on the body roentgenogram

Reference points

Ba : The most posteroinferior point of the occipital condyle
C2ip : The most inferoposterior point of the second cervical vertebra
C4mp : The mid-posterior point of the fourth cervical vertebra
C7ip : The most inferoposterior point of the seventh cervical vertebra
Gi : The most inferior point of the angular process of the mandible
H : The most inferoanterior point of the hyoid body
L2mp : The mid-posterior point of the second lumbar vertebra
Me : The most inferior point of the mandibular symphysis
S4ia : The most inferoanterior point of the fourth sacral vertebra
S4ia′ : Intersection from the point S4ia perpendicular to the body axis (X)
So : Intersection between the inferior border of the basisphenoid and the tympanic bulla
T2mp : The mid-posterior point of the second thoracic vertebra

Reference lines

C (Cervical line) : The line C2ip-C7ip
MP (Mandibular plane) : The line Me-Gi
PCBP (Posterior cranial base plane) : The line Ba-So
X (Body axis) : The horizontal line through point C2ip

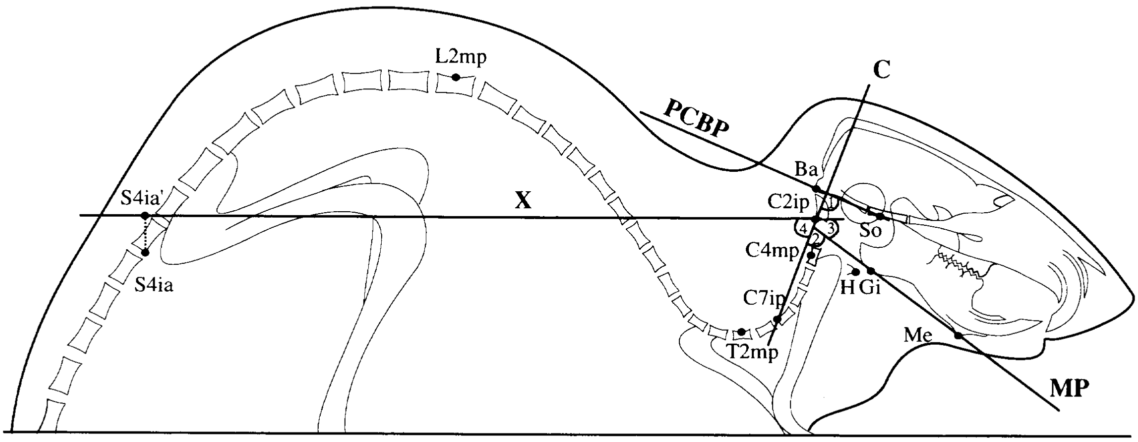


Fig 2 Reference points and lines used for analysis from the body radiographs abbreviations we shown in Table 1

were fixed rigidly onto a craniostat with a pair of ear rods to orientate their median plane vertically. The distance from the focus of the X-ray beam to the median plane of the head was 18 cm for both LA and AX, and that from the median plane of head to the film was 6 cm for LA and 3 cm for AX respectively. A steel wire of 10 mm was attached to each film for calibration and films were enlarged 4.8 times when printed on photographic paper.

Postural and cephalometric analyses

postural analysis was carried out according to methods previously described for humans by Borden and Rechtman[17], Ishihara[18], Opdebeeck[19]. Reference points and lines were shown in Table 1 and Fig. 2, with postural variables in Table 2. The position of the hyoid bone was analyzed only at the end of experiment.

Cephalometric analysis, including craniofacial sizes, shapes and area of capsular matrix elements, was modified from Hanada[20], Engström[21], Ito, *et al.*[22] and Moss[23]. Reference points and planes were shown in Table 3 and Figs. 3a, 3b, with linear and angular variables in Tables 4 and 5, and functional capsular matrix elements in Fig. 4. All measurements were analyzed from tracings of spines and craniofacial structures with a digitizer (MYPAD-A3 Logitec Co., Japan) interfaced to a personal

Table 2 Linear and angular variables of the body posture

Linear variables
CC : Cervical curvature : Distance from C4mp to the line C
TC : Thoracic curvature : Distance from T2mp to the line X
LC : Lumbar curvature : Distance from L2mp to the line X
SL : Spinal length : Distance from C2ip to the point S4ia′

Angular variables
∠1 (CCA) : Craniocervical angle : ∠Line C/PCBP
∠2 (CMA) : Cervicomandibular angle : ∠Line C/MP
∠3 (PMA) : Posturomandibular angle : ∠Line X/MP
∠4 (CI) : Cervical inclination : ∠Line C/Line X

Hyoid position*
H to Me : Hyoid position relative to mandible : Distance from H to the point Me
H to line C : Hyoid position relative to cervical column : Vertical distance from H to the Line C
H to So (Ver) : Hyoid vertical position relative to cranium : Vertical distance from H to the point So
H to So (Hor) : Hyoid horizontal position relative to cranium : Horizontal distance from H to the point So

* : Analyzed only at the end of experiment

computer (EPSON PC-286L-STD-N, Japan).

As body radiographs were taken twice, the mean value was calculated from the two images for each variable. Statistical differences were evaluated using ANOVA test. When a significant F valus was noted, Student-Newman-Keuls test was continued. Statistical significance was set at $p \leqq 0.05$.

Methodological errors

Methodological errors (standard errors of mean difference) of the postural analysis were calculated using the mean values of all 80 rats, whilst cephalometric analysis was evaluated from the mean values from 10 normal rats taken four times with four-hour intervals. The largest standard errors were 0.63 for the postural analysis and 0.62 for the cephalometric analysis.

Results

Alterations of body and muscle weight

During the experiment, some rats died due to anesthesia accident (three rats in groups E1, E2 and N) and daily animal care (four rats in group E2). As cylindrical cages for E2 were tilted upwards at 45°, rats tended to rush upwards from the cages before covering the food box over it. The head of running animal was injured coincidentally with the food box. The final numbers at the end of experiment in each group were N : 19, C : 20, E1 : 19, E2 : 15.

Body weight increased constantly in all groups (Fig. 5). At the end of experiment, significant differences in body weight were found between N (407.9 g) and other three groups (C : 354.4 g, E1 : 339.2 g, and E2 : 339.0 g), but no significant differences were found among the later three groups. As a consequence, all comparisons in the present study were made only between C and E1 or E2.

Fig. 6 showed the proportional weight of craniocervical muscles to body weight for the three groups at the end of experiment. In group E1, the ratio of MA and MH to body weight was significantly smaller compared with group C animals. In group E2, the ratios of TM, SH, SM and LC to body weight increased and that of MH decreased compared with group C. The ratios to body weight were larger in MA, MH, SH and SM in E2 compared with E1, which indicated that group E2 had generally greater mass of craniocervical muscles.

Alterations of body and head postures

Table 6 and Fig. 7 showed the postural changes that occurred in experimental rats compared with control rats throughout the experiment. E1 and E2 showed significantly reduced thoracic and lumbar

Table 3 Reference points on the cephalometric roentgenogram

Lateral cephalogram	
Co	The most posterior point of the mandibular condyle
E	Intersection between the frontal bone and the most superoanterior point of the posterior limit of the ethmoid bone
Gp	The most posterior point of the angular process of mandible
Id	The most superoanterior point of the labial alveolar process on the mandibular incisor
In	The most posterior point on the external occipital protuberance
Li	The incisal edge of the mandibular incisor
Ma	Intersection between the maxillary alveolar process and the mesial surface on the maxillary first molar
Ma′	Intersection between the mandibular alveolar process and the mesial surface on the mandibular first molar
Md	The point on the distal cusp of the mandibular third molar
Mm	The point on the mesial cusp of the mandibular first molar
Mn	The deepest point of the antegonial notch
Mp	Intersection between the maxillary alveolar process and the distal surface of the maxillary third molar
Mp′	Intersection between the mandibular alveolar process and the mesial surface of the mandibular third molar
N	The frontonasal suture point
Na	The most anterior point of the nasal bone
Pns	The most posterior point of the hard palate
Pr	The most inferoanterior point of the labial alveolar process on the maxillary incisor
Pr′	The most inferoanterior point of the lingual alveolar process on the maxillary incisor
Ui	The incisal edge of the maxillary incisor
Axial cephalogram	
Cp (Cp′)	The tip of the coronoid process
Ea (Ea′)	The center point of the lateral margin of the external auditory aperture
Or (Or′)	The deepest point on the orbital margin
Sq (Sq′)	Intersection between the line through the intershenoidal synchondrosis (Which is perpendicular to the sagittal line) and the posterior margin of the squamous bone
Zy (Zy′)	Intersection between the line through the intersphenoidal synchondrosis (which is perpendicular to the sagittal line) and the zygomatic arch

* : Ba, Gi, Me, So : same as in Table 1 ; Co (Co′), GP (GP,) : same as in the lateral cephalogram

curvatures (TC, LC) and increased spine length (SL), indicating a flattened back. The craniocervical and cervicomandibular angles (∠CCA, ∠CMA) were larger and posturomandibular angle (∠PMA) and cervical inclination (∠CI) were smaller, which were typical features of head extension. The smaller distances from H to Me and larger one from H to Line C indicated a whole backward movement of the cervical column, the hyoid bone and the mandible, as a consequence of flattened beck and head extension.

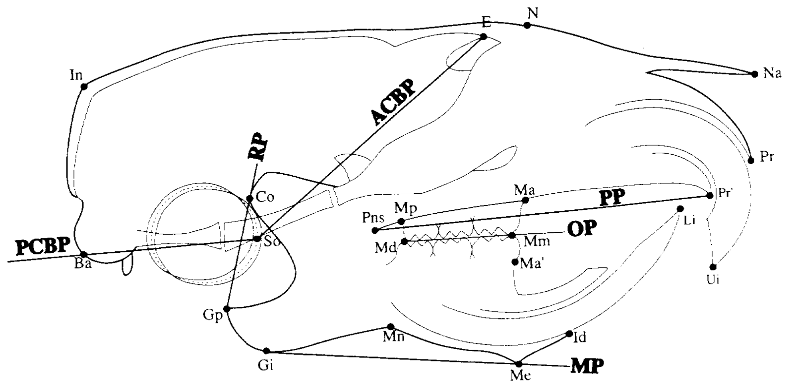
Alterations of craniofacial growth

Tables 7, 8 were craniofacial sizes and shapes represented by linear and angular variables in the three groups at 55 and 85 days of age respectively, Tables 9, 10 and Figs. 8, 9 illustrated the alterations of craniofacial sizes and shapes of the experimental groups as compared to the control group.

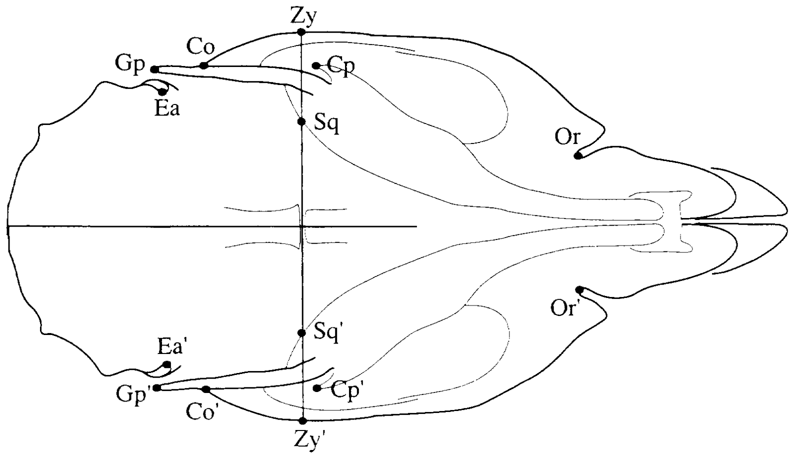
1. Comparison of craniofacial sizes and shapes of E1 and C

1) Linear variables

Craniofacial skeletal dimensions were significantly smaller in E1 than C, especially in anterio-posterior length. At 55 days of age, the cranial length (Na-In, Pr-Ba), the neurocranial length (E-In), the viscerocranial length (Pr-E, Pr'-E), the interior premaxillary length (Pr'-MA), the palatal length (Pr'-Pns), the anterior viscerocranial height (Ma-E) and the bizygomatic width (Zy-Zy') were smaller in E1 than group C animals. But the maxillary incisor-molar distance (Ui-Ma) was smaller and mandibular incisor-molar distance (Li-Ma') was larger in E1 than group C animals. In addition at the



a : On the lateral cephalogram



b : On the axial cephalogram

Fig 3 Reference points and planes used for analysis from the cephalometric radiographs
Abbreviations were shown in Table 3

end of experiment, the ramus height (Co-Gp) decreased but the maxillary incisal alveolar thickness (Pr-Pr') increased (Table 7, 9).

2) Angular variables

In contrast with the significantly smaller craniofacial dimensions, there were few shape alterations in E1. The alterations only included an upward rotation of the neurocranium, shown by the smaller cranial base angle (∠Ba-So-E), a larger cranial vault angle (∠In-E-So), increased angle between the neurocranial height and cranial vault (∠Ba-In-E), a smaller premaxillary angle (∠Pr-E-So, ∠Pr'-E-So) with retroinclined incisors (∠Ui-E-So, ∠Ui-Pr-Ma, ∠Li-Id-Ma') in both jaws and a sharp antegonial notch angle (∠Gi-Mn-Me) (Tables 8, 10).

Alterations in growth of the craniofacial skeleton in terms of both size and shape in E1 at the end of the experiment were illustrated in Fig. 8.

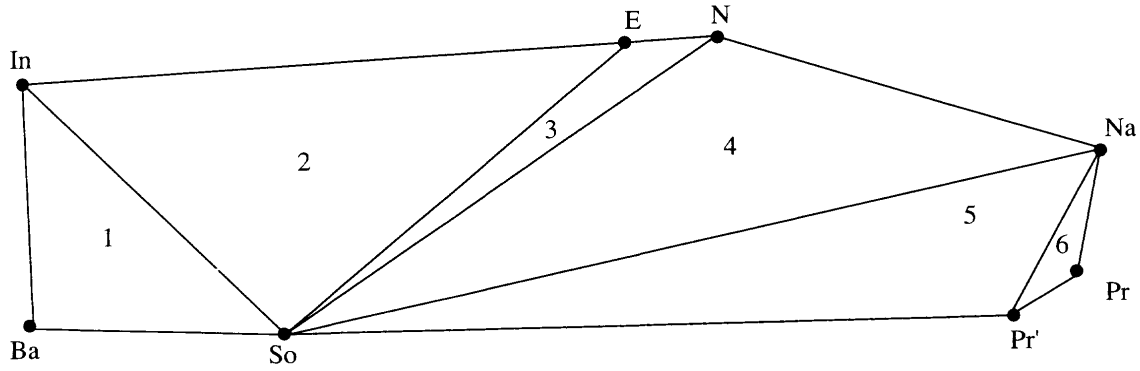
2. Comparison of craniofacial sizes and shapes of E2 and C

1) Linear variables

The craniofacial sizes of E2 were very similar to group C, except for the decreased cranial lengths (Na-In, Pr-Ba), neurocranial length (E-In) and exterior viscerocranial length (Pr-E) at 55 days of age and decreased mandibular incisor-molar distance (Li-Ma') at 85 days of age (Table 7, 9).

2) Angular variables

Almost no shape alteration was found in E2 animals until 55 days of age apart from a smaller maxillary incisal angle (∠Ui-E-In). However at 85 days of age, significant changes occurred in all items of viscerocranium investigated, *i. e.,* the nasal bone angles (∠Na-N/In-E, ∠Na-N/so-E), the premaxillary angles (∠Pr-E-In, ∠Pr'-E-In, ∠Pr-E-So, ∠Pr'-E-So) and the maxillary incisor angles (∠Ui-E-In, ∠Ui-E-So) were smaller relative to both the cranial vault and the anterior cranial base,



Area 1: cerebellum and midbrain element
Area 2: cerebral and olfactory element
Area 3: frontocribriform element
Area 4: upper nasal element
Area 5: lower nasal element
Area 6: external nasal element

Fig 4 A diagram of the capsular matrix elements established from lateral cephalograms

Table 4 Linear variables of the craniofacial complex

Lateral cephalogram
Na-In : Superior cranial length
Pr-Ba : Inferior cranial length
So-E : Anterior cranial base length (Plane, ACBP)
So-Ba : Posterior cmanial base length (Plane, PCBP)
E-In : Neurocranial length
Ba-In : Neurocranial height
Pr-E : Exterior viscerocranial length
Pr′-E : Interior viscerocranial length
Ma-E : Anterior viscerocranial height
Mp-E : Posterior viscerocranial height
Na-N : Nasal bone length
Pr-Ma : Exterior premaxillary length
Pr′-Ma : Interior premaxillary length
Pr′-Pns (Mp)* : Palatal length (Plane, PP)
Pr-Pr′ : Maxillary incisor alveolar thickness
Ui-Ma : Maxillary incisor-molar distance
Co-Id : Mandibular length
Gp-Id : Mandibular body length
Co-Gp : Ramus height (Plane, RP)
Li-Ma′ : Mandibular incisor-molar distance
Axial cephalogram
Or-Or′ : Inter orbital width
Cp-Cp′ : Bicoronoid width
Zy-Zy′ : Bizygomatic width
Sq-Sq′ : Viscerocranial width
Co-Co′ : Bicondylar width
Ea-Ea′ : Neurocranial width
Gp-Gp′ : Bigonial width

* : Pns and Md were obscure at the beginning of experiment, thus substituted by Mp and Md′ (the second molar) respectively

Table 5 Angular variables of the craniofacial complex

∠Ba-In E : Neurocranial height to cranial vault
∠Ba-So-E : Cranial base angle
∠In-Ba-So : Neuhocranial height to posterior cranial base
∠In-E-So : Cranial vault angle
∠Na-N/In-E : Nasal bone to cranial vault
∠Pr-E-In : Exterior premaxilla to cranial vault
∠Pr′-E-In : Interior premaxilla to cranial vault
∠Ui-E-In : Maxillary incisor to cranial vault
∠Na-N/So-E : Nasal bone angle
∠Pr-E-So : Exterior premaxilla angle
∠Pr′-E-So : Interior premaxilla angle
∠Ui-E-So : Maxillary incisor angle
∠Ui-Pr-Ma : Maxillary incisor inclination
∠PP/E-So : Palatal plane angle
∠OP/E-So : Occlusal plane angle
∠MP/E-So : Mandibular plane angle
∠OP/PP : Occlusal plane to palatal plane
∠MP/PP : Mandibular plane to palatal plane
∠MP/OP : Mandibular plane to occlusal plane
∠RP/MP : Gonial angle
∠Gi-Mn-Me : Antegonial notch angle
∠Li-Id-Ma′ : Mandibular incisor inclination

while the palatal plane angle (∠PP/E-So) and the mandibular plane angle (∠MP/E-So, ∠MP/OP) were larger. These alterations indicated a significant downward rotation of the upper viscerocranium and the mandible in E2. The unique alteration in neurocranium was the larger angle between neurocranial height and cranial vault (∠Ba-In-E) Table 8, 10).

Alterations in growth of the craniofacial skeleton in terms of size and shape in E2 at the end of experiment were illustrated in Fig. 9.

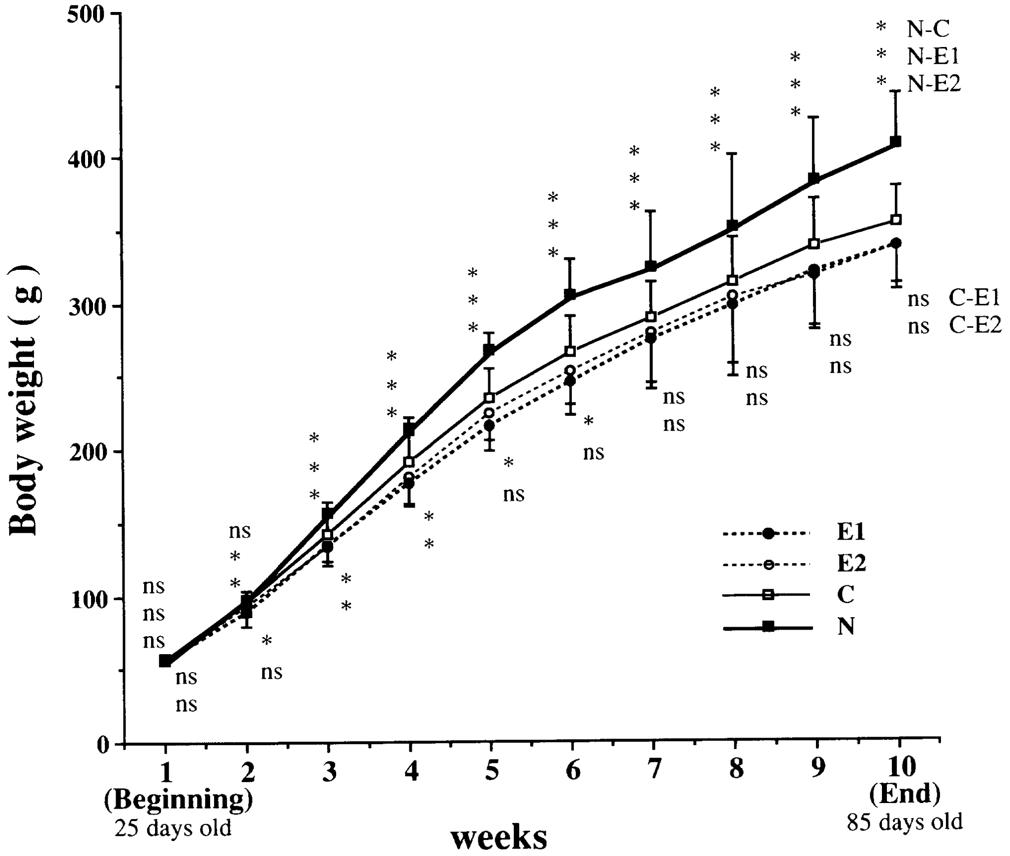


Fig 5 Comparison of body weight (g) between normal group (N) and control group (C) or experimental group (E1 and E2) during whole experimental period recorded once a week

There were significant differences between N and C or E1 or E2, but no statistical differences were found between C and E1 or E2 at the end of experiment

3. Comparison of craniofacial sizes and shapes of E1 and E2

El animals showed generally smaller craniofacial dimensions than E2, such as the viscerocranial length (Pr-E, Pr'-E,) and height (Ma-E), the incisor-molar distance in both jaws (Ui-Ma, Li-Ma') and the bizygomatic width (Zy-Zy') at 55 days of age. At the end of experiment the superior cranial length (Na-In), the premaxillary length (Pr-Ma, Pr'-Ma) and the palatal length (Pr'-Pns) were also significantly smaller in El animals compared with E2 animals (Table 7, 9).

However, E2 showed a significant downward rotation of the upper viscerocranium and mandible compared with El, shown by smaller premaxillary angles (∠Pr-E-In, ∠Pr'-E-In) and maxillary incisal angle (∠Ui-E-In) and larger mandibular plane angles (∠MP/E-So) at 55 days of age. The rotation increased by the end of experiment as shown by the smaller nasal vault (∠Na-N/In-E, ∠Na/So-E) and exterior premaxillary angles (∠Pr-E-So) and the larger palatal and mandibular plane (∠PP/E-So, ∠MP/OP) angles. El animals showed an upward rotation of the neurocranium, shown by the larger cranial base angle (∠Ba-So-E), smaller cranial vault angle (∠In-E-So) and smaller maxillary incisor inclination (∠Ui-Pr-Ma) compared with E2 animals (Table 8, 10).

4. Comparison of areas of the capsular matrix elements

Table 11 showed the areas of capsular matrix elements in the three groups at the end of experiment. Areas of the frontocribriform and upper nasal elements (elements 3 and 4) were significantly larger in E2 than in both C and El.

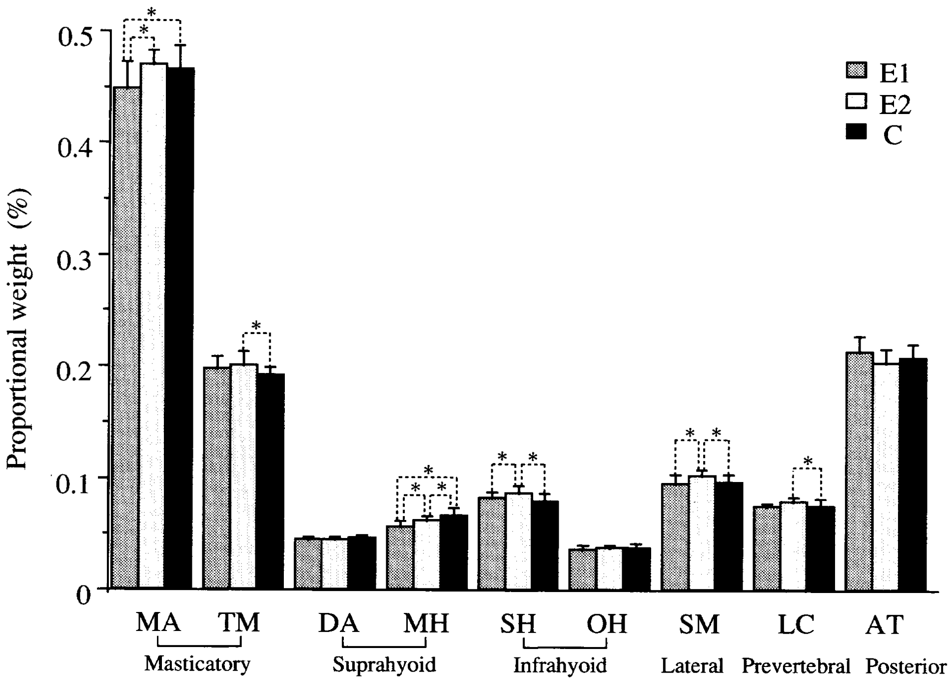


Fig 6 A graph to show the proportional weight of different craniocervical muscles to body weight for the three animal groups (E1, E2 and C) at the end of the experiment

Comparing E1 animals with C group animals, demonstrated relatively smaller values of muscles for MA and MH. Similar comparisons for E2 showed relatively greater values for TM, SH, SM and LC but smaller value for MH compared with C. Comparing E1 with E2 animals showed relatively increased values for MA, MH, SH and SM in E2 than E1

Discussion

Effects of head extension on craniofacial growth

The relationship between head posture and craniofacial morphology has been previously studied by Björk[24]. He observed that individuals with flattened cranial base and a retrognathic facial type carried their heads in extended position. Solow and Tallgren[7] found an association between the above two factors that individuals with head extension in relation to their carvical columns had an increased lower anterior facial heights, an obtuse gonial angles, mandibular retrognathism and decreased vertical dento-alveolar development. This association was agreed by many studies in which individuals with a head extension and an elevated face display the characteristics of skeletal open bite[9,25] or a steep mandibular plane and the prominent chin[10]. However, effects of head extension on craniofacial growth have not been tested by animal experiments.

In the present study, a long-term head extension of rats was induced during their growing period to investigate how does head posture affect the craniofacial growth. The general growth of animals in E1, E2 and C was almost equally affected within the restricted cages, compared to group N. On the contrary, the craniofacial growth in E1 and E2 was strongly influenced in some different manners. Head extension in horizontally raised animals (E1) affected both craniofacial dimensions and dento-alveolar position and shapes, whereas head extension with a 45° uprighted body orientation (E2) affected mainly the craniofacial shapes (Fig. 10).

Altered skeletal dimensions induced by head extension in E1 reflected chiefly in the craniofacial lengths, which may relate to much higher sagittal growth rates than the vertical or the frontal one in rats. The ratardation of growth in craniofacial size has been significant up to 55 days of age, which indicated that craniofacial growth in size was influenced easily in the earlier period with the rapid growth and the relatively large dimensional

Table 6 Body and head postures in experimental and control groups at the middle and the end of experiment

	Group / Items	E1 Mean±SD	E2 Mean±SD	C Mean±SD	F value	E1 : C	E2 : C	E1 : E2
Middle								
Linear	CC	1.13±0.26	1.19±0.20	1.02±0.23	2.74			
	TC	10.18±1.27	9.88±1.48	12.24±1.52	16.03***	*	*	ns
	LC	14.14±2.56	15.98±1.86	21.30±2.81	45.75***	*	*	ns
	SL	110.50±5.49	108.42±3.79	100.39±4.54	25.97***	*	*	*
Angular	∠CCA	109.99±9.88	112.78±8.61	97.77±8.15	15.82***	*	*	ns
	∠CMA	98.52±7.44	101.06±8.64	84.16±8.20	25.00***	*	*	ns
	∠PMA	36.70±5.45	36.12±5.88	41.91±7.37	8.49***	*	*	ns
	∠CI	46.99±8.30	44.71±9.44	53.65±9.65	5.07**	*	*	ns
End								
Linear	CC	1.22±0.21	1.36±0.28	1.18±0.29	2.27			
	TC	10.63±1.71	10.96±1.43	12.64±1.66	8.61***	*	*	ns
	LC	13.89±2.76	14.67±2.67	26.11±3.02	56.75***	*	*	ns
	SL	129.20±6.20	126.41±6.77	117.32±5.82	19.24***	*	*	ns
Angular	∠CCA	109.70±8.58	111.93±9.78	102.41±7.29	6.27**	*	*	ns
	∠CMA	95.93±8.82	98.85±8.08	88.41±7.83	7.68**	*	*	ns
	∠PMA	36.23±8.03	33.96±9.79	44.81±5.90	9.65***	*	*	ns
	∠CI	42.55±5.89	41.20±5.09	46.85±7.41	3.98*	*	*	ns
Hyoid	H to Me	16.24±0.60	16.07±0.64	16.74±0.65	5.62**	*	*	ns
	H to Line C	12.88±0.64	12.96±0.62	12.01±0.68	12.15***	*	*	ns
	H to So (Ver)	7.54±0.60	7.43±0.60	7.54±0.45	0.23			
	H to So (Hor)	1.20±0.46	1.14±0.43	0.94±0.45	1.79			

(E1 : C, E2 : C, E1 : E2 = Statistical significance)

Unit : linear variable (mm), angular variable (degree). Abbreviations were shown in Table 2
ANOVA test : F value * : $p<0.05$ ** : $p<0.01$ *** : $p<0.001$
Student-Newman-Keuls test was made when a significant F value in ANOVA test was noted and statistical significance was set at $p \leqq 0.05$ level

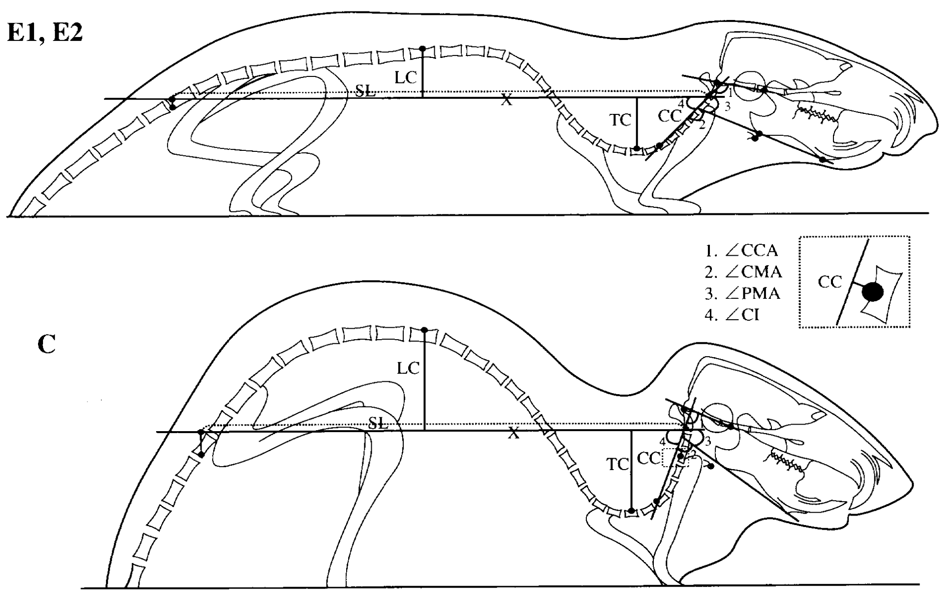


Fig 7 Posture changes of the experimental rats (E1, E2) compared with control rats (C)
In the constructed cages of E1 and E2, the animals backs were flattened shown by the reduced thoracic and lumbar curvatures (TC, LC) and increased spinal lengths (SL). As a consequence of this, head extension was induced, represented by the larger craniocervical (∠CCA) and cervicomandibular (∠CMA) angles, combined with both smaller posturomandibular (∠PMA) angle and cervical inclination (∠CI)

Table 7 Craniofacial sizes represented by linear variables at the middle and the end of experiment

	Items / Group	E1(N=20) Mean±SD	E2(N=19) Mean±SD	C(N=20) Mean±SD	F value	E1(N=19) Mean±SD	E2(N=15) Mean±SD	C(N=20) Mean±SD	F value
	LA	Middle of experiment (55 days old)				End of experiment (85 days old)			
Neuro-cranium: Cranial length	Na-In	245.61±6.03	247.86±4.28	252.46±5.67	8.37***	263.43±4.42	267.88±3.44	268.95±5.01	8.34***
	Pr-Ba	235.40±5.07	237.10±3.64	240.43±6.11	5.11**	257.69±4.10	260.40±3.91	262.11±4.98	4.96*
Neuro-cranium	So-E	105.44±2.95	105.73±2.31	106.82±2.43	1.59	110.75±3.40	110.69±1.93	110.95±1.79	0.05
	So-Ba	57.65±1.74	58.12±1.85	58.76±2.23	1.63	63.53±1.59	64.71±2.05	64.65±1.96	2.34
	E-In	144.15±3.57	145.48±3.53	148.42±4.00	6.95**	148.96±4.15	150.49±3.48	152.55±3.21	4.77*
	Ba-In	58.20±1.75	58.03±1.97	58.92±1.91	1.25	59.25±1.45	59.25±1.43	59.30±2.09	0.01
Upper viscerocranium	Pr-E	107.05±3.03	109.05±2.96	110.57±3.21	6.61**	119.87±2.73	123.41±3.10	121.99±4.20	4.61*
	Pr′-E	100.14±2.64	102.23±2.34	103.97±3.06	10.10***	110.08±2.36	114.66±3.32	112.92±3.78	8.95***
	Ma-E	59.79±1.52	60.95±1.32	61.28±1.57	5.65**	65.35±1.54	66.86±1.77	66.82±2.07	5.56**
	Mp-E	69.23±1.41	69.92±1.45	69.20±1.67	1.39	72.11±1.38	72.62±1.22	72.91±1.79	1.02
	Na-N	85.56±3.36	86.71±3.06	86.90±3.39	0.97	97.29±2.17	98.98±3.51	98.16±3.31	1.32
	Pr-Ma	81.04±2.51	82.35±2.87	82.97±2.70	2.68	90.28±2.41	92.10±2.23	92.72±2.76	4.94*
	Pr′-Ma	65.30±2.23	66.82±2.80	67.97±2.52	5.63**	72.14±2.86	74.92±1.98	75.52±2.19	10.74***
	Pr′-Pns	118.67±3.26	119.87±1.93	121.16±3.42	3.50*	125.97±2.21	129.01±2.28	129.16±2.40	11.30***
	Pr-Pr′	18.69±1.17	18.85±0.67	18.38±0.92	1.26	20.55±0.58	20.10±0.92	19.85±1.16	3.05**
	Ui-Ma	71.34±2.49	73.89±2.80	74.23±2.21	7.92***	79.11±3.02	83.09±2.74	84.07±2.28	18.21***
Mandible	Co-Id	116.94±3.66	116.99±2.16	118.11±3.53	0.84	127.16±3.15	128.40±2.13	128.16±3.07	0.95
	Gp-Id	120.14±3.70	120.04±2.45	120.74±3.70	0.25	130.59±3.22	131.10±2.08	131.49±2.76	0.51
	Co-Gp	25.60±1.21	25.56±0.87	26.26±1.16	2.58	31.02±1.23	31.79±1.61	32.04±1.17	5.06**
	Li-Ma′	63.82±2.20	65.95±1.91	65.20±1.82	5.83**	68.11±2.19	71.21±2.48	73.23±1.94	26.89***
	AX								
	Or-Or′	36.99±1.01	37.37±1.10	37.83±1.62	2.21	41.91±0.99	41.81±0.93	42.25±1.01	1.02
	Cp-Cp′	94.00±1.53	94.94±1.58	95.10±2.36	2.02	100.94±1.94	101.01±1.70	100.89±3.35	0.01
	Zy-Zy′	113.05±1.86	115.08±1.60	115.08±2.91	5.58**	123.07±2.42	123.98±2.11	124.05±2.85	0.87
	Sq-Sq′	50.98±4.49	51.37±5.09	52.75±5.59	0.67	78.74±2.38	78.48±3.65	78.55±2.03	0.04
	Co-Co′	96.59±1.43	97.33±1.58	97.54±2.60	1.29	101.81±1.86	102.04±2.95	102.42±2.54	0.31
	Ea-Ea′	74.85±1.69	75.76±1.36	75.75±1.54	2.27	79.65±1.53	79.56±1.69	80.25±1.45	1.10
	Gp-Gp′	94.83±2.76	95.15±1.84	95.36±3.09	0.21	101.38±2.29	102.06±2.96	102.56±2.17	1.13

Unit : mm (×4.8). Abbreviations were shown in Table 4. * : $p<0.05$ ** : $p<0.01$ *** : $p<0.001$

changes.

Whilst skeletal shape modifications in E2 involved mainly in the viscerocranium and were not significant until the rats reached 55 days of age, indicating that shape modifications were progressive and cumulative. These findings seemed to agree Spence's demonstration[26] that the growth of neurocranium in rats is almost completed by 70 days, while that of viscerocranium continues to develop.

Colton[27] noted only a smaller cranial base length in his bipedal experiments, in the present study, however, smaller dimensions were identified not only in the craniofacial length but also in its height and width in E1. Rotations of the viscerocranium[12], the cranial base and the neurocranium in its anterior part[28] have been reported in bipedal rats, whilst both the upward rotation of neurocranium (E1) and the downward rotation of upper viscerocranium and mandible (E2) were found in the present study. The large palatal and the mandibular plane angles and the retroinclined incisor position found in the present study seemed to support the clinical observations described above.

Effects of head extension on the craniocervical muscles

Several experiments have demonstrated that altered muscular function can influence the craniofacial morphology[29~31] and suggested that altered muscle recruitment may be a plausible explanation for the skeletal changes[32].

In the present study, the muscle weight was used to evaluate its volume alteration, as one of indicators for the functional muscular activity following the altered head posture and body orientation. In E2, the proportional weight were significantly larger in most craniocervical muscles, such as the

Table 8 Craniofacial shapes represented by angular variables at the middle and the end of experiment

	Items (Group)	E1 (N=20) Mean±SD	E2 (N=19) Mean±SD	C (N=20) Mean±SD	F value	E1 (N=19) Mean±SD	E2 (N=15) Mean±SD	C (N=20) Mean±SD	F value
		Middle of experiment (55 days old)				End of experiment (85 days old)			
Neuro-cranium	∠Ba-In-E	90.00±1.89	90.07±2.35	88.95±2.15	1.72	95.17±0.99	95.44±1.49	93.99±1.65	5.57**
	∠Ba-So-E	143.11±1.69	144.57±1.81	145.35±1.76	8.41**	141.39±1.60	142.85±2.06	143.78±2.01	7.85**
	∠In-Ba-So	92.05±2.26	91.21±2.51	91.78±2.12	0.67	88.62±1.70	87.49±1.69	88.39±1.76	1.96
	∠In-E-So	34.85±0.90	34.13±1.01	33.92±1.01	5.00**	34.82±1.20	34.24±1.23	33.86±1.22	3.07
Upper viscerocranium	∠Na-N/In-E	158.30±1.73	156.93±1.17	157.65±2.23	2.91	159.78±1.74	157.05±2.11	159.97±2.65	8.82***
	∠Pr-E-In	148.27±1.54	147.04±1.28	147.34±1.71	3.46*	149.61±1.58	147.61±1.71	149.81±2.38	6.32**
	∠Pr′-E-In	138.72±1.32	137.52±1.11	138.19±1.92	3.18*	140.61±1.70	138.90±1.74	141.20±2.40	5.92**
	∠Ui-E-In	128.65±1.26	127.73±1.13	128.55±1.17	3.47*	130.56±1.80	129.03±2.12	131.40±2.64	4.88*
	∠Na-N/So-E	123.47±1.56	122.78±1.34	123.73±2.08	1.62	124.95±1.54	122.82±2.21	126.11±2.29	11.26***
	∠Pr-E-So	113.43±1.37	112.91±1.40	113.40±1.53	0.81	114.79±1.49	113.38±1.66	115.94±2.01	9.21***
	∠Pr′-E-So	103.87±1.09	103.41±1.10	104.27±1.66	2.08	105.79±1.61	104.66±1.67	107.34±1.94	10.31***
	∠Ui-E-So	93.80±1.08	93.60±1.12	93.59±0.99	0.25	95.75±1.47	94.78±1.95	97.54±2.24	9.47***
	∠Ui-Pr-Ma	61.65±1.50	63.63±1.32	64.94±1.40	11.18***	61.13±2.04	64.32±1.87	65.03±2.44	17.53***
	∠PP/E-So	37.22±1.19	38.52±1.04	37.89±1.42	2.41	36.50±1.02	38.49±1.83	36.51±1.73	8.94***
Mandible	∠OP/E-So	39.38±1.62	39.73±2.68	39.79±2.06	0.21	36.63±1.89	38.03±1.96	37.64±1.95	2.47
	∠MP/E-So	48.20±1.60	50.06±1.83	49.14±1.54	6.10**	47.01±1.31	49.98±1.77	47.32±1.32	20.54***
	∠OP/PP	2.42±1.20	2.70±1.60	2.57±1.44	0.19	1.32±0.92	1.41±1.18	1.63±1.18	0.40
	∠MP/PP	11.59±1.04	12.12±1.37	11.50±1.51	2.91	11.02±1.18	11.49±0.93	10.82±1.42	2.75
	∠MP/OP	8.81±1.57	10.34±2.77	9.51±2.33	2.24	10.41±1.96	11.94±1.69	9.69±1.48	7.45**
	∠RP/MP	75.75±3.10	76.25±2.85	76.33±3.29	0.21	74.96±3.00	76.49±2.92	74.93±2.44	1.69
	∠Gi-Mn-Me	158.30±2.54	159.32±2.53	159.08±2.27	0.94	157.09±2.33	158.36±1.79	158.92±2.29	3.52*
	∠Li-Id-Ma′	79.90±2.75	80.27±2.87	79.77±3.21	0.15	76.15±3.06	77.32±1.89	79.09±2.80	5.92**

Unit : degree, Abbreviations were shown in Table 5. * : p<0.05 ** : p<0.01 *** : p<0.001

Table 9 Statistical significance of craniofacial sizes represented by linear variables between each two groups at the middle and the end of experiment

Items	E1 : C	E2 : C	E1 : E2	Items	E1 : C	E2 : C	E1 : E2
	Middle of experiment (55 days old)				End of experiment (85 day old)		
Na-In	*	*	ns	Na-In	*	ns	*
Pr-Ba	*	*	ns	Pr-Ba	*	ns	ns
E-In	*	*	ns	E-In	*	ns	ns
Pr-E	*	ns	*	Pr-E	ns	ns	*
Pr′-E	*	*	*	Pr′-E	*	ns	*
Ma-E	*	ns	*	Ma-E	*	ns	*
Pr′-Ma	*	ns	ns	Pr-Ma	*	ns	*
Pr′-Pns	*	ns	ns	Pr′-Ma	*	ns	*
Ui-Ma	*	ns	*	Pr′-Pns	*	ns	*
Li-Ma′	*	ns	*	Pr-Pr′	*	ns	ns
Zy-Zy′	*	ns	*	Co-Gp	*	ns	ns
				Ui-Ma	*	ns	*
				Li-Ma′	*	*	*

Abbreviations were shown in Table 4. Statistical significance was set at p≦0.05 level by student-Newman-Keuls test, when a significant F value in ANOVA test was noted

masticatory (TM), infrahyoid (SH), the lateral (SM), and the prevertebral (LC). This may attribute to the fact that head extension was weakened to some degree by altering body orientation. Such an upward tilted body orientation may alter the craniocervical muscular balance and their func-

Table 10 Statistical significance of craniofacial shapes represented by angular variables between each two groups at the middle and the end of experiment

Items	Statistical significance			Items	Statistical significance		
	E1 : C	E2 : C	E1 : E2		E1 : C	E2 : C	E1 : E2
	Middle of experiment (55 days old)				End of experiment (85 day old)		
∠Ba-So-E	*	ns	*	∠Ba-In-E	*	*	ns
∠In-E-So	*	ns	*	∠Ba-So-E	*	ns	*
∠Pr-E-In	ns	ns	*	∠Na-N/In-E	ns	*	*
∠Pr′-E-In	ns	ns	*	∠Pr-E-In	ns	*	*
∠Ui-E-In	ns	*	*	∠Pr′-E-In	ns	*	*
∠Ui-Pr-Ma	*	ns	*	∠Ui-E-In	ns	*	ns
∠MP/E-So	ns	ns	*	∠Na-N/So-E	ns	*	*
				∠Pr-E-So	*	*	*
				∠Pr′-E-So	*	*	ns
				∠Ui-E-So	*	*	ns
				∠Ui-Pr-Ma	*	ns	*
				∠PP/E-So	ns	*	*
				∠MP/E-So	ns	*	*
				∠MP/OP	ns	*	*
				∠Gi-Mn-Me	*	ns	ns
				∠Li-Id-Ma′	*	ns	ns

Abbreviations were shown in Table 5. Statistical significance was set at p≦0.05 level by student-Newman-Keuls test, when a significant F value in ANOVA test was noted.

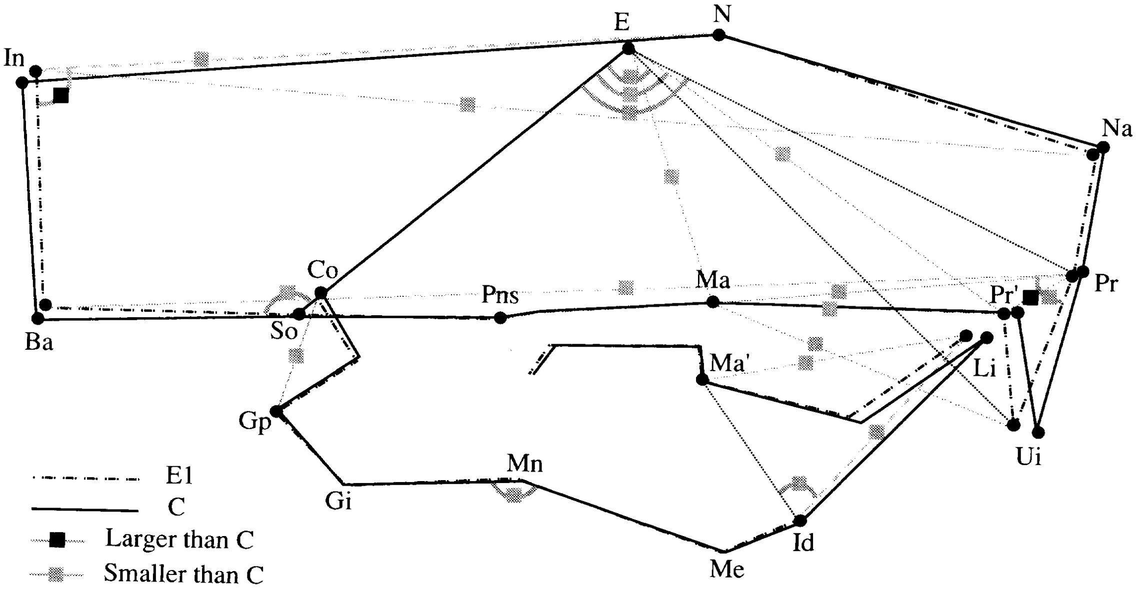


Fig 8 A trace of average E1 lateral cephalogram superimposed onto that of average C group animal at 85 days of age, using So-E as the reference plane, to show alterations in the size and shape of the craniofacial skeleton during growth
E1 animals had smaller craniofacial lengths and heights than C, retroinclined maxillary and mandibular incisors with the neurocranium rotated upwards

tional activities. It suggest that altered body orientation induced the compensatory adaptation of the muscles. This part would be discussed in detail by further histochemical and electromyographic analyses.

Possible factors involved in biological mechanism for craniofacial changes

Skeletal growth and form depend on a number of interacting factors which could be explained by the soft tissue stretching hypothesis[11] and the func-

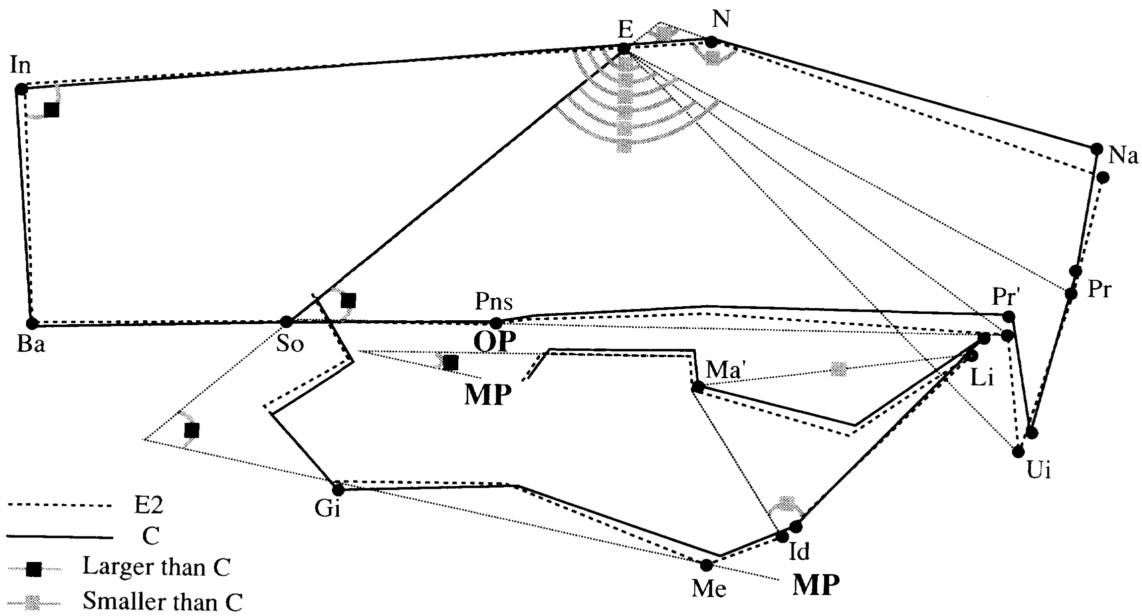


Fig 9 A trace of average E2 lateral cephalogram superimposed onto that of average C group animal at 85 days of age, using So-E as the reference plane, to show alterations in the size and shape of the craniofacial skeleton during growth

E2 animals showed almost the same craniofacial sizes as C, but had a pronounced downward rotation of the upper viscerocranium and mandible

Table 11 Area of capsular matrix elements in the three groups and their comparisons each other at the end of experiment

Group / Items	E1 Mean±SD	E2 Mean±SD	C Mean±SD	F value	Statistical significance E1 : C	E2 : C	E1 : E2
Element 1	1880.82± 68.61	1914.54± 77.63	1915.57± 96.29	1.07			
Element 2	4706.36±164.25	4682.94±137.85	4712.07±133.68	0.18			
Element 3	566.59± 85.64	690.44±121.27	600.93± 77.93	7.53***	ns	*	*
Element 4	4881.95±213.61	5103.56±274.13	4868.76±302.40	4.00*	ns	*	*
Element 5	3485.05±145.30	3549.54±196.70	3643.48±270.78	2.73			
Element 6	227.11± 29.12	205.37± 23.38	216.20± 28.03	2.68			

Unit : mm^2. Definition of elements was shown in Fig. 4.
ANOVA test : F value * : $p<0.05$ ** : $p<0.01$ *** : $p<0.001$
Student-Newman-Keuls test was made when a significant F value in ANOVA test mas noted and statistical significance was set at $p\leqq 0.05$ level

tional matrix hypothesis[33]. The former put the emphasis on the passive traction of the surrounding soft tissues as an indicator of bone growth. Whilst the latter claimed any skeletal growth in size, shape and spatial position is accomplished by functional matrix activities, in which the periosteal matrix is to alter the bone form, and the capsular matrix is to alter capsular volume[34]. This adaptive response of the craniofacial skeletal complex to functional changes has been observed by removing muscle or denervation of the masticatory muscles[35~37]. The effects of periosteal tension to stimulate bone deposition and mechanical lording to alter bone shape has been demonstrated experimentally[38,39].

In the present study, a retardation of craniofacial growth in sizes were observed in E1 animals (Fig. 8). The stretching of craniocervical muscles caused by head extension may genrate a strain force on the cranium to restrain its forwerd development, and increase the periosteal tension which directly acted on the skeletal units by osseous deposition or resorption to alter the size and shape of craniofacial skeleton. Furthermore, the tension force from whole craniocervical musculature passing between

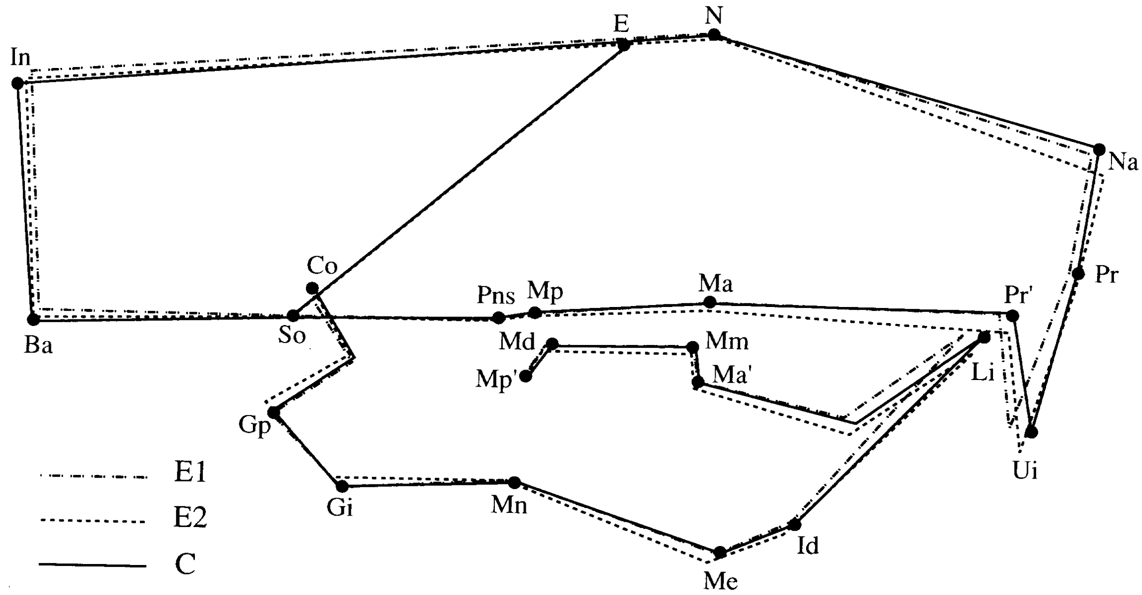


Fig 10 A trace of average E1, E2 lateral cephalograms superimposed onto that of average C group animal at 85 days of age, using So-E as the reference plane, to show alterations in the size and shape of the craniofacial skeleton during growth

E1 animals had smaller craniofacial sizes than controls, retroinclined maxillary and mandibular incisors and an upward rotation of the neurocranium. E2 animals had almost the same craniofacial sizes as controls, but had a downward rotation of the upper viscerocranium and mandible

the shoulder girdle, the hyoid and the mandible acted on the mandible, then the cervical colum, hyoid bone and manible were displaced backwards, and the shape of the mandible was modified, showing a sharp anteogonial notch. However, there was no firm evidence in the present study to explain the retroinclined incisors, although it was assumed to be an altered periosteal matrix activity according to the definition of periosteal matrix of Moss[33].

In E2 animals, a retardation of the usual extent of upwards viscerocranial rotation relative to the early developed neurocranium, with the larger areas of the frontocribriform and the upper nasal elements, was observed (Fig. 8 and Table 11). The tilted body orientation might alter the activities of whole craniocervical musculature to maintain such a body orientation, then capsular matrix activity appeared to be dominant than periosteal one. This capsular matrix activity may indirectly act on the functional cranial components by a passive translation of these components to alter the volume of capsule.

On the other hand, the tilted body orientation in E2 animals might reflexively alter the visual line and the carriage of head because the carriage of head is adjusted by equilibrium sensations, controlled by both visual and otolith organs. Consequently a compensatory rotation of the otic capsule occurred, combined with a retardation of the viscerocranial rotation. Moss[12] has demonstrated that rotation of the otic capsule correlated with either an accentuation or a retardation of viscerocranium following altered head and body position, and stated the physiologic basis of such otic capsule movement was primarily related to the need for reorientation of the utricular maculae.

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