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Synthetico-analytic Approach to Mechanism of Shaping of the Brain through Simulation with 3D Computer Graphics

Setsuya Fujita

Department of Pathology, Kyoto Prefectural University of Medicine, Kawaramachi Hirokoji, Kamikyoku, Kyoto 602, Japan

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At the beginning of development of the central nervous system(CNS), the anlage of the brain and spinal cord is a tiny tube-like structure (neural tube) which is solely composed of matrix cells. Previous studies on matrix cell kinetics have revealed that there are systematic local gradients in the rate of matrix cell proliferation in the wall of the neural tube. Shaping of the brain may be accomplished by the local difference in growth rates. This idea is tested by simulation with 3D computer graphics. The neural tube composed of 60 segments is created in computer. Growth of each segment is calculated by a Gompertzian growth function. Growth of human brain can be simulated by selecting an optimal set of the retardation factors, Lambda(I), of 60 segments. Analysis of the simulation indicates that growth of matrix cells at different rates is a determining factor of brain morphogenesis. Using different sets of Lambda(I), formation of brains in rhesus monkey, rat, mouse is studied and it is concluded that evolution of shapes of mammalian brains may be explained by changing kinetics of matrix cell systems.

Introduction

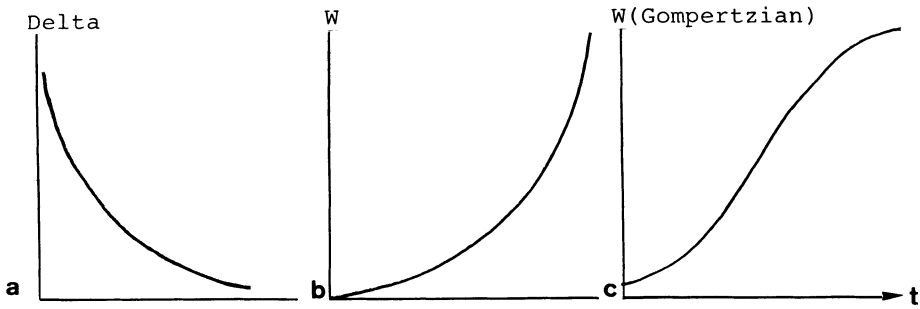
Morphogenesis is one of the most exciting phenomena in living systems. An embryo develops from a single cell, fertilized egg, and grows into an adult form. Striking changes in shape and size do take place during this ontogeny.

Why and how such and such particular forms are created repeatedly with remarkable precision in the world of multicellular organisms, is the question that has occupied minds of many biologists since the time of Aristotle.

The morphogenesis during the ontogeny is, in general, closely related to proliferation of cells and growth of the tissues they constitute. There are, however, exceptions; in the earliest stage of development of many animals, cells increase in number only dividing the volume of egg cytoplasm. In amphibians, this process continues until the neural plate develops and rolls up to form a neural tube. A.G.Jacobson and Gordon (1) analyzed this phenomenon with an elegant experiment by computer simulation. This is, however, the quite exception. The morphogenesis during vertebrate ontogeny is observed as a process evolved through growth of the animal.

Mehnert(1898)(2) having studied growth curves, found that the growth rates, being high at the beginning, decreases steadily as the development proceeds (Fig 1, a). When this growth rate reaches a very low level, the increase in size ceases (Fig. 1, c).

Figure 1



Schmalhausen(3) described this relationship differently by an equation (cf. Fig. 1, b for this graph.)

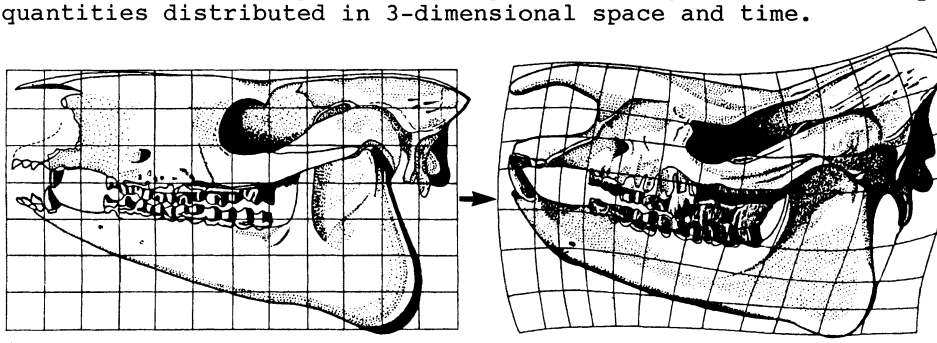
$$W=at^3 \qquad .....(1)$$

where W=size of the animal or an organ, a=const and t=time. Specific growth rate, Delta, is calculated by

$$Delta=\frac{1}{W}\cdot\frac{dW}{dt}=\frac{3at^2}{at^3}=\frac{3}{t} \qquad .....(2)$$

Following Mehnert's pioneer work, many investigators studied quantitative aspects of growth, measuring weight or length of various organs and organisms, and analyzed them theoretically. Allometric equation was used frequently in this analysis.

These investigations were quantitative, but have paid little attention to the form or pattern-representation of the organs or organisms. Most investigators of this kind of research were originally interested in morphogenesis and many of them declared that their principal subject of the study was developmental shape changes in organs or individual animals. Nevertheless, they failed to address directly to this problem. It was because there were no ways of approaching a problem of growth, to analyze quantities distributed in 3-dimensional space and time.



Hirachius agrarius Aceratherium tridactylus

Figure 2 (Adapted from D'Arcy Thompson, 4)

In this respect, a complementary study was commenced by D'Arcy Thompson in 1917(4). He invented a method to analyze morphological changes of living organisms as a transformation of original form into a deformed coordinate. Fig. 2 is an application of his method to a process of evolution of head bones of extinct ungulates from Hirachius agrarius to Aceratherium tridactylus. He applied this method to many examples and succeeded to demonstrate that changes in form can be described by first transforming the original (Cartesian) coordinates into curvilinear coordinates and by subsequent mapping of the original figures into the latter.

His approach, however, lacked quantitative treatment to express this kind of transformation. He realized this shortcoming very well and remarked that "When the system becomes no longer orthogonal,....., then the transformation is no longer within the reach of comparatively simple mathematical analysis", and added in the final section of his treatise that "If the difficulty of the description could be overcome, it is by means of such coordinates in space that we could at last obtain an adequate and satisfying picture of the processes of deformation and the directions of growth." (4)

The classical researchers of growth since Mehnert have focused their attention exclusively on quantitative aspects of developing organs and organisms in terms of weight or length and abandoned analysis of shape. On the other hand, studies of D'Arcy Thompson introduced non-orthogonal curvilinear coordinate system to represent changes in forms, and disregarded, although reluctantly, quantitative treatment of the transformation.

Thanks to introduction of computer as a graphic tool, it becomes now feasible to fuse these 2 approaches into a new technology suitable for quantitative analysis of forms of developing living systems changing in time and space. Transformation of figures into any coordinate systems can be computed and represented on computer CRT, either in 2-dimensional or in 3-dimensional graphics.

The present paper is dealing with a description of this method and its application for investigation of morphogenesis of the human, monkey and mouse brains.

Materials and Methods

Development of CNS is particularly suitable for this type of computer analysis. In all the vertebrates, including humans, development of CNS starts with a small single tubular anlage, called neural tube. The wall of the neural tube is composed solely of matrix cells(5) at the onset of neurogenesis (Fig.3).

In analyzing cytokinetics of matrix cells, it has been found that their generation times not only steadily increase in length as the development proceeds(6), but also differ locally(7). Rapidly expanding regions of the brain showed shorter generation times in comparison with slowly growing parts, thereby indicating that difference in local cell production rate may create a force to deform original Cartesian coordinates into non-orthogonal curvilinear ones in the sense of D'Arcy Thompson.

The neural tube appears to be a suitable organ to test this idea for the following reasons.

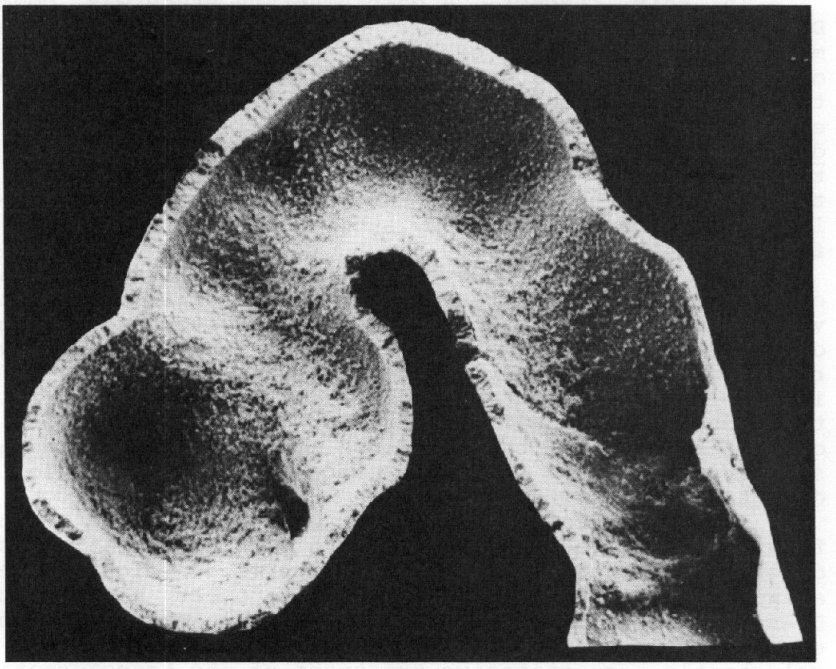


Figure 3
Mouse neural tube at embryonic day 10
X 50

1) The neural tube is composed solely of matrix cells and devoid of intercellular substances, as shown in Fig.3. 2) Size of a matrix cell does not change significantly during development. 3)Thickness of the wall of neural tube increases in parallel with the increase in the surface area, so that growth of a minute volume within the wall of neural tube may be regarded as homogeneously expanding in all directions. So the neural tube is selected as material of the present investigation.

At first, following D'Arcy Thompson, Cartesian coodinate is drawn in the space which includes the neural tube (Fig. 4). Three-dimensional coordinate values of each lattice point i (xi, yi, zi) are determined and written into an array in computer program. In total, number of the lattice points were 298.

The computer used in the present simulation experiment is Hewlett-Packard 32 bit desktop computer (HP9520) equipped with a 40 MB hard-disk unit.

The neural tube in the computer comprizes 60 segments which were further subdivided into 558 triangular patches (Fig. 4) for hidden surface removal and smooth surface rendering in computer graphics. The subdivision of the surface of neural tube is illustraed in Fig. 4, in wire-frame representation in 30 times magnification (left), and in surface model representaion in 150 times enlargement (at right). For presentation of 3D grapahics, the second generation machine (7, Fig. 5) of the Color Image Analyzer(CIA, 8, 9) was used in the present experiment. CIA is an intelligent color terminal, equipped with 2 complete sets of frame-buffers of [512x512(pixels)x8(bits)x4(boards)+512x512x1 (flag board)], and controlled by "macro-commands" written in ROM(read-only-memory) incorporated in the terminal.

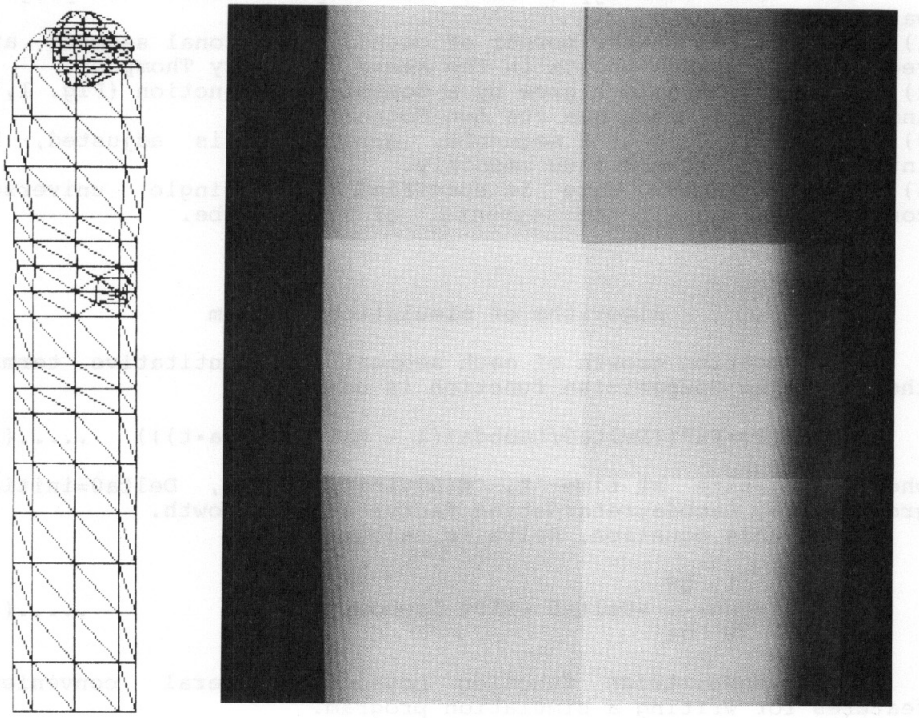


Figure 4

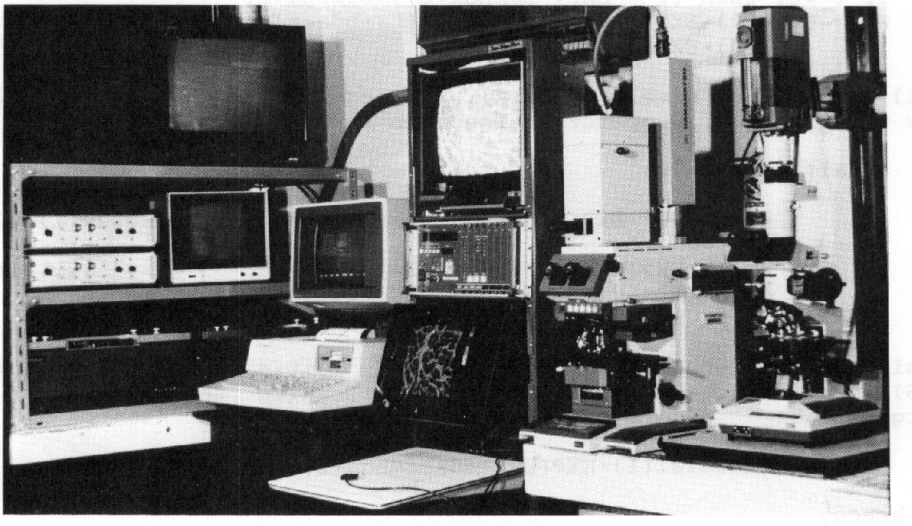


Figure 5

The basic strategy to write the computer simulation program was as follows.
1) The 8 points at the corner of each 3-dimensional segment are regarded as lattice points in the sense of D'Arcy Thompson.
2) These lattice points grow by a Gompertzian function (Fig. 1, a and c). Each segment has its own Gompertzian.
3) At the junction of 2 segments, growth rate is adjusted, by interpolation, to continue smoothly.
4) Initial growth rate is specified as a single universal constant through all the segments of neural tube.

Algorithm of simulation program

To describe growth of each segment in quantitative terms, the following Gompertzian function is used.

$$W(t)=W(0)\cdot EXP((Delta0/Lambda)(1 - EXP(-Lambda\cdot t))) \quad .....(3)$$

where W(t)=size at time t, W(0)=initial size, Delta0=initial growth rate, Lambda=retardation factor of the growth.
From this equation, Delta is calculated by

$$Delta(t)=\frac{1}{W}\cdot\frac{dW}{dt}=Delta0\cdot EXP(-Lambda\cdot t) \quad .....(4)$$

The Gompertzian function possesses several convenient features for writing a simulation program.
i) Initial portion of the growth curve of the Gompertzian is similar to an exponential growth function at the growth rate Delta0. Because,

$$\text{if } t\sim 0, \text{ then } (1 - EXP(-Lambda\cdot t))=Lambda\cdot t,$$
$$W(t\sim 0)=W(0)\cdot EXP((Delta0/Lambda)(Lambda\cdot t))$$
$$=W(0)\cdot EXP(Delta0\cdot t) \quad .....(5)$$

ii) The maximal size, Final(I), of segment i that is attainable by the growth of Gompertzian function is given by

$$Final(I)=\lim_{t\to\infty} W(t)$$
$$=\lim_{t\to\infty} W(0)\cdot EXP((Delta0/Lambda(I))(1- EXP(-Lambda(I)\cdot t)))$$
$$=W(0)\cdot EXP(Delta0/Lambda(I)) \quad .....(6)$$

since $\lim_{t\to\infty} EXP(-Lambda(I)\cdot t)=0$.

Using these expressions, Final(I) and Initial(I)= W(0), equation (6) is rewritten for each segment i, with the argument,

$$Final(I)=Initial(I)\cdot EXP(Delta0/Lambda(I))).$$

Thence,

$$Lambda(I)=Delta0\cdot LOG(Initial(I)/Final(I)) \quad .....(7)$$

iii) The curve of a Gompertzian function is unequivocally specified if Delta0, Initial(I), and Lambda(I) are given. Delta0 is a universal constant and Initial(I), which is the initial size of segment i, can be regarded also as a single common constant. It is right to conclude that the growth of segment i solely depends on the constant Lambda(I) for that segment i. Lambda(I) is calculated by the equation (7).

Actual computation of the growth of neural tube was made in an incremental fashion with a discrete time interval Δt.

$$W(t + \Delta t)=W(t) + \frac{dW}{dt} \cdot \Delta t \qquad .....(8)$$

From eq.(2)

$$\frac{1}{W} \cdot \frac{dW}{dt}=Delta, \qquad \frac{dW}{dt}=Delta \cdot W(t)$$

$$W(t + \Delta t)=W(t)+ Delta \cdot W(t) \cdot \Delta t=(1+ Delta \cdot \Delta t) \cdot W(t) \qquad .....(9)$$

Therefore, replacing t with discrete time, Day, counted from the onset of development of CNS, size of a segment i at the next day, i.e. (Day+1), was calculated incrementally,

$$W(I,Day+1)=(1 +Delta(I,Day))(W(I,Day)) \qquad ....(10)$$

Three-dimentional coordinate values of growing human neural tube were calculated by taking cube root of W(I,Day+1) of eq.(10).

Gompertzian functions with suitable set of Delta0 and Lambda(I) could reproduce size changes of neural tube during development successfully to a certain extent. However, initial bendings of mesencephalic and cervical flexures are not realized by the growth rate difference in the Gompertzian alone, so that an additional constraint to fix the distance between hypothalamus and rostro-ventral aspect of rhombencephalon is introduced for the earliest period of development(10).

Fig. 6 is lateral views of the wire-frame representation of human embryonic brains drawn on CRT by this method. The pictures were directly copied from the CRT.

3D graphic simulation of brain morphogenesis

The surface model of the neural tube is composed of 558 triangular patches listed, in an array Tri(k,3), in order of xk, yk, zk. Normal vectors were calculated using these elements in each line of the array, to perform hidden surface removal and smooth shading by Gouraud's procedure under naturally looking illumination vectors.

Growth of the neural tube in 3D graphics is represented in Figure 7 in 6 times enlargement. Pictures of this Figure roughly correspond to those depicted in the first column of Fig. 6, but in different magnification, and the brains are slightly rotated around Y-axis. In Fig. 7, a represent initial condition of the neural tube at 24 days of pregnency. It grows into b at 26 days postconception, c at 28 days, d at 35 days, e at 38 days, f at 41 days, g at 46 days, and h at 54 days of pregnancy.

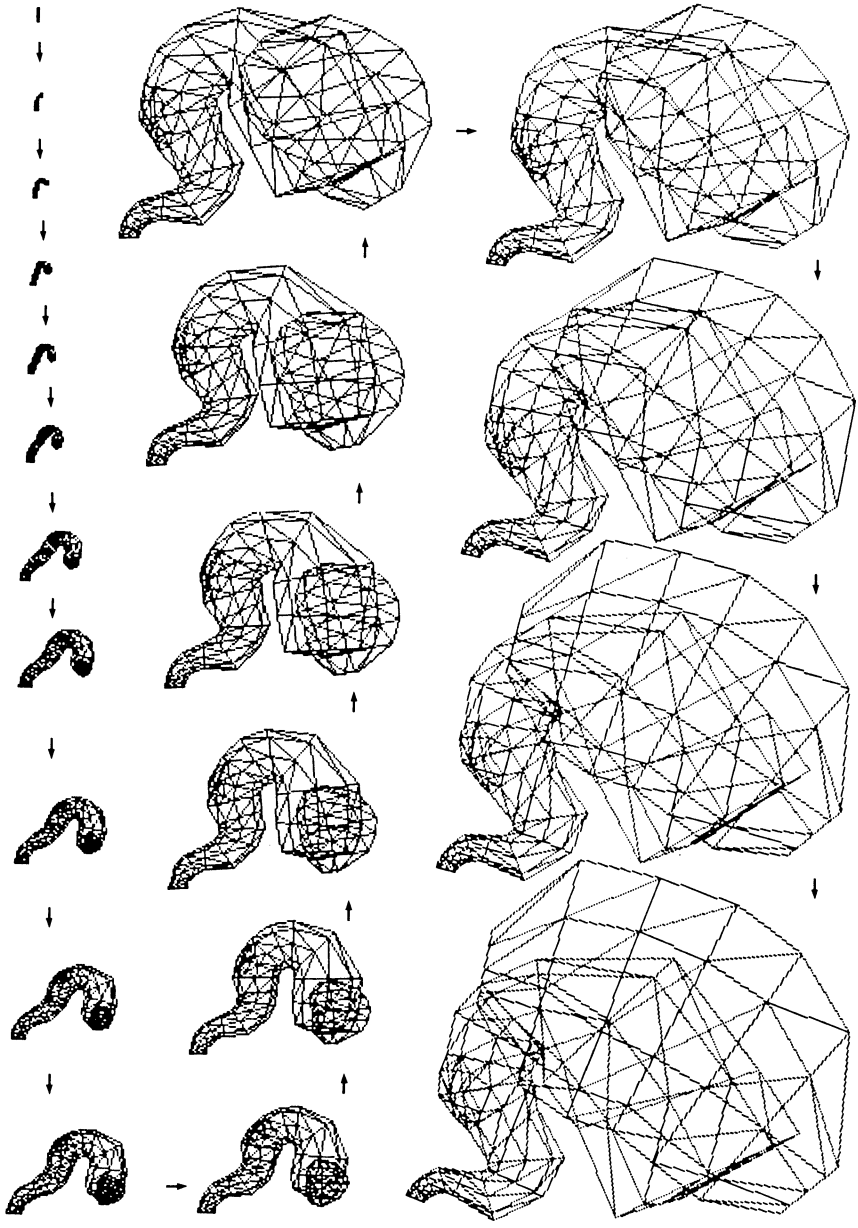


Figure 6

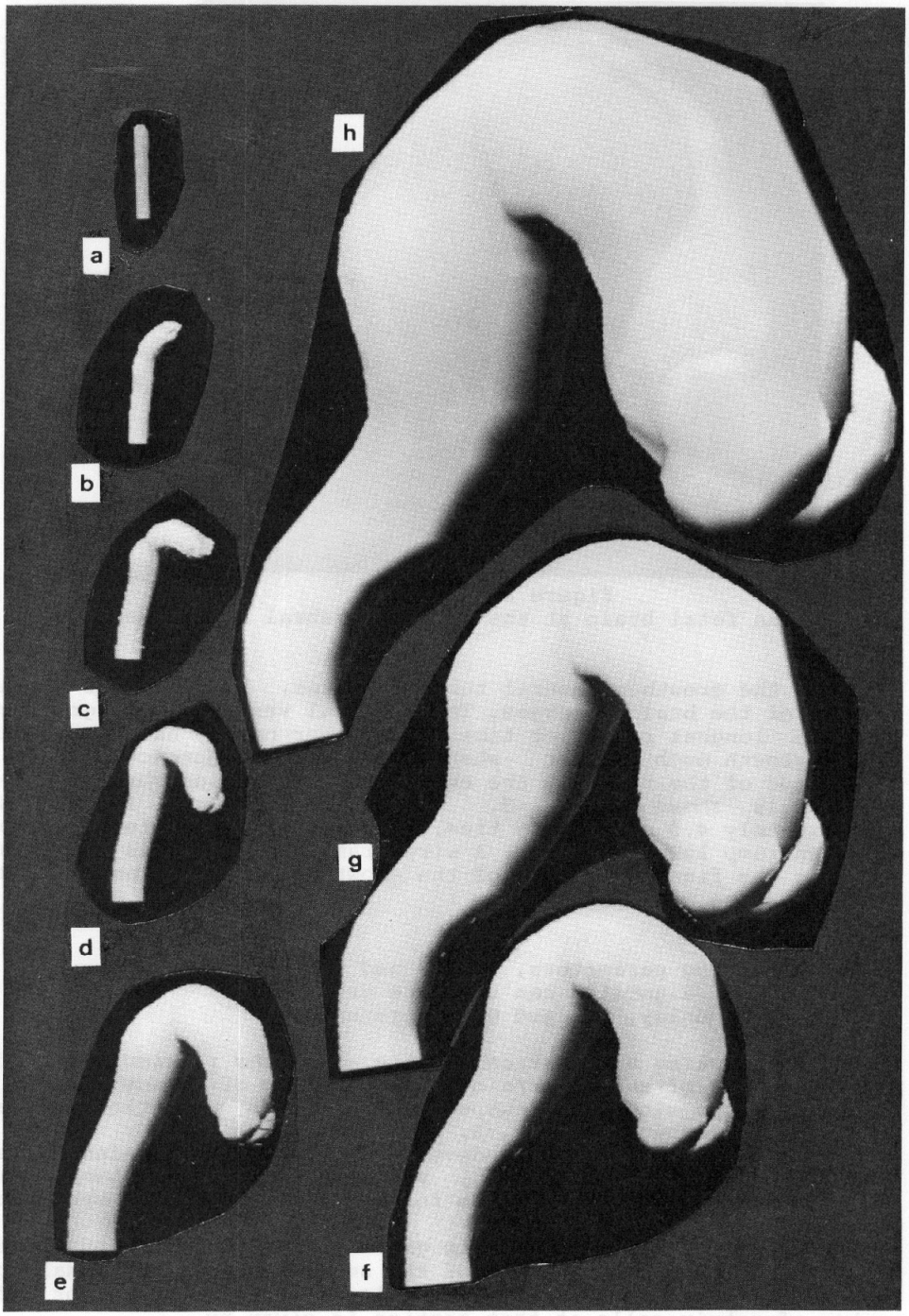


Figure 7 3D graphics of developing neural tube

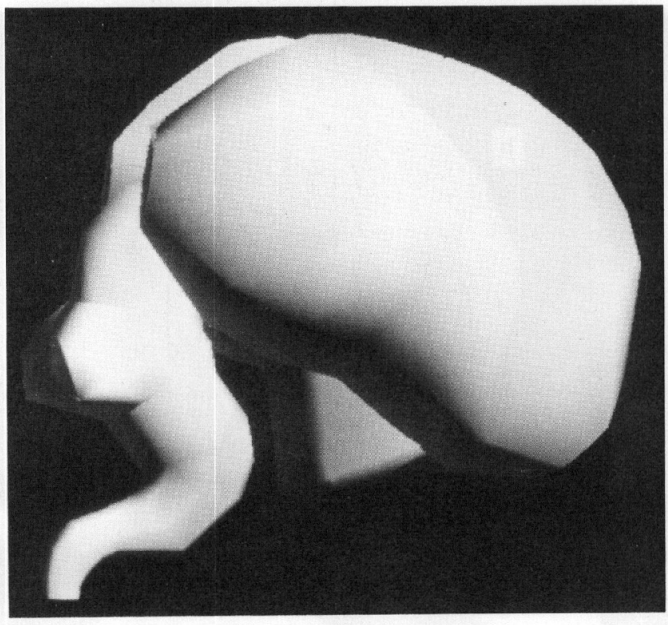


Figure 8
Human fetal brain at the end of cerebral neuronogenesis

As the growth of neural tube continues, the size of each portion of the brain enlarges. The cerebral vesicle keeps growing for the longest period of time while other parts have stopped their growth much earlier when each Delta is approaching to 0. The shape of the brain at the end of stage II of cerebral hemispheres is shown in Fig. 8. The fronto-occipital length is approximately 4.5 cm at this time, corresponding to a fetal stage of crown-rump length of 10cm. A wire-frame model of this stage is shown as the final condition of the growth in Fig. 6, and Fig. 8 is its surface model.

By varying parameters, Delta0 and Lambda(I), the same algorithm can simulate growth of monkey, rat and mouse brains

An interesting application of this algorithm is simulation of the production of smaller brains such as those of rhesus monkey, rat and mouse within the respective period of gestation. The result is represented in Fig. 9, a, b and c.

In order to simulate the development of smaller brains like those of monkey, rat or mouse, values of Delta0 and Lambda(I) have to be modified from those for human brains in a specific manner as discussed below.

1) The initial value of specific growth rate, Delta0, must be larger to meet faster growth of the smaller brains in smaller animals. Since doubling time of volume of a brain part or of number of matrix cells at that portion is given by

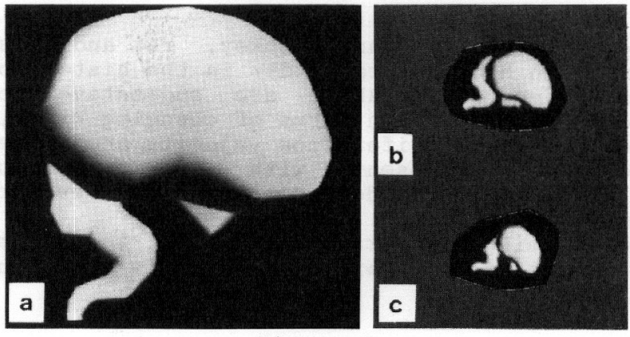


Figure 9
Fetal brains at the end of cerebral neuronogenesis
a: rhesus monkey, b:rat, c:mouse. At the same magnification as Fig. 8.

$$\text{Doubling time}=(\text{LOG}(2))/\text{Delta}(I,\text{Day}) = (\text{LOG}(2))\cdot\text{EXP}(\text{Lambda}(I,\text{Day}))/\text{Delta0}, \quad ....(11)$$

the fact mentioned above can be interpreted that the doubling time of the matrix cell population in smaller brains is shorter than that in the human brain.
2) Secondly, the same simulation experiment reveals that values of Lambda(I,Day) of corresponding segment i and of the same Day is always in order of mouse>rat>monkey>human, thereby indicating faster retardation of the growth in the smaller brains. This fact seems to correspond to shorter gestational time in which development of the brain is completed in the smaller animals.

Discussion

In the present simulation experiment, it is shown that different rates of growth at different loci can create complicated shape of brain in human, monkey, rat and mouse fetuses. Changing intermediate shapes and sizes of the developing neural tube in the computer graphics are compared with real brains at various stages of development, and it becomes evident that the similitude is more satisfactory than we have expected. A Gompertzian function or its slightly modified form simulates nicely the real growth of respective segment of the brain, if the 2 parmeters Delta0 and Lambda(I) are adequately specified.

By application of the simulation technique for human and non-human brain morphogenesis, it becomes evident that the larger brains start their development with much slower growth rate (i.e. longer generation times of matrix cells) but that decay of the growth rate is also slower. As a result of this slower growth, the brain is formed taking longer time but meanwhile matrix cells producing much greater number of cells(i.e. neurons), since decay of the growth rate does not take place so quickly. On the contrary, small brains are formed by faster growth at the beginning followed by faster decay of matrix cell proliferation, so that smaller and simpler brains are produced in a shorter period of time.

Although brains of human, monkey, rat and mouse are by no means arranged in hierarchical order in the history of evolution, the findings mentioned above are suggestive to interpret evolution of human brains in terms of changing kinetics of matrix cells; Decrease in proliferation velocity and in decay rate of the proliferation, represented with a specific set of Delta0 and Lambda(I) seem to play a determinant role in evolution of the human brain.

A study aiming at verifying direct relationship between matrix cell kinetics and brain morphogenesis is now under way.

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8-4
Q: I would like to congradulate you of this model, which I feel is a very correct one. However, I would like to point out that the regulation of cell proliferation is very complicated. To put this regulation into activity at a defined location at a defined time period of development, it needs a large armamentarium. This armamentarium is coded in the DNA of the developing animal. This means that reasonably big genetic differences between species can be expected, and they need not be limited to genes determining the brain mass only, but also to genes determining the location of changes and topography of microstructure. Do you agree?
(Y. Collan)

A: I completely agree with you, Dr. Collan, in recognizing the importance of regulation in proliferation of the matrix cells that constitute the developing CNS. It is the difference in genetic expression in cell proliferation and differentiation in space and time that really produces different brains in different species of animals. Those differences had appeared formidably complicated and defying our effort to understand the mechanism of morphogenesis in different animals. The present investigation, however, reveals that only small differences in growth rates of corresponding segments of brains can produce remarkably different shapes of brains in various animals. Accordingly, we can infer that genetic changes during evolution of the brain may be small and stepwise, and hopes are opened up that we may find an approach to understand the mechanism of evolution of the human brain in terms of cell proliferation and gene mechanism to regulate that proliferation.

Q: Was the growth parameter the brain volume? If you regard the brain surface as the parameter, then is there possibly another growth kinetics or more remarkable interspecies differences? (S. Eins)

A: For the first question, the answer is yes. I calculated regional differences in volume growth. But, if you take cube root of the volume, it represents growth in length, and if you then take its square, it is the surface area. There is no theoretical difference in taking these 3 parameters in the present simulation.

Q: I congratulate you on this excellent presentation on brain growth by computer graphics simulation.
Would you please commment on the differences if any, between human and animal intelligence as related to the brain structure. (E. Hall)

A: It is a difficult question.
At least 2 factors appear to be important to determine sophisticated functions of the brain, especially in the celebrum, 1) Quality of indivisual neurons that form the neural circuits, and 2) total number of neurons in the brain region, provided that the neuroglial tissue renders optimal circumstances. The importance of the quality of neurons has been stressed and most investigations of brian function has focused their attention to this feature of neurons. Another factor, number of neurons, may be, I believe, more important than the quality of neurons. It is especially true among species in which the latter factor is determined by the same or very similar set of genes. In most human microencephaly, there are severe deficiency in intelligence. No humans with one forth or less of the normal size of the brains can speak nor understand language properly. The brain of rhesus monkey remains on this level. So I infer that, at the final stage of the primate evolution, the increase in brain volume or in number of neurons should have played an essential role in promoting the intelligence of the mankind. If this inference is true, a chimpanzee or orang-outang, after one or two doubling of their brain size would aquire astonishing ability to speak language and understand abstract thought as all the present

day humans do. I don't know how to verify this prediction by an actual experiment, though.

Q: Do you take in account the effect of epigenic cell death upon the final form and function of the brain? Jacobson has demonstrated the overproduction of neuroblasts for motoneurones in the frog - where maybe 70% of the cells will die. Changeaux has put forward the hypothesis of "selective stabilization" in which epigenetic cell death plays a major part in the final form of the mature CNS. Would you care to comment? (V. Howard)

A: In the present simulation, I only calculated overall growth of each segment of the brain. I have been very much interested in the cell death problem since it was first pointed out by Levi Montalcini. However, there is no kinetic study on cell death in the developing CNS to be incorporated in growth analysis. If such study were carried out, it would be interesting to take that factor into consideration in dynamic analysis of brain morphogenesis to make the analysis more precise.
But as for the present study, it treats only overall volume increase in various parts of the brain. If cell death has occured to a certain extent, it had only lessened the apparent growth rate of that part and had no effect on the calculation in computer simulation.