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Developmental and Age-related Changes in Morphometry and Cellular Density of the Vestibular Ganglion in the Chick

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Abstract

Vestibular ganglia at different stages of development in the chick and that of the adult were fixed in formal-saline. Serial sections were taken and stained by cresyl fast violet to analyse the results. Cells were categorised and counted. Ganglionic volume is the greatest on E18 during the whole ontogeny even though its rostrocaudal length is the greatest in the adult. This is quite different from that observed in other ganglia studied where the ganglionic volume is the greatest in the adult situation. The increased size of the ganglion on E18 might be related to the increased size of the cells, increased size of the neuropil (network of neuronal processes and neuroglia) and to the effects of tissue reactions resulting from the toxic substances of the dead cells. The loss of cells in the adult in comparison to that observed on the day of hatching might indicate a functional reduction as a result of ageing process. The fluctuation in the number of cells during development might be considered as a normal process for the purpose of re-arrangement for better organisation in order to perform an efficient function. The cell-loss has essentially ended around E15 by the time the ganglion begins to show its greatest expansion so as to reach its maximum size on E18. The appearance of a few light cells in the vestibular ganglion on E6 might indirectly indicate the beginning of an early establishment of a functional connection. This might possibly mean that an early development of functional organisation of the vestibular ganglion is an important factor for the proper development of other organs and systems during embryonic development. The appearance of an increased number of tiny cells in the adult ganglion is peculiar from that observed in other ganglia studied; possibly these tiny cells, by their growth, maturation and establishment of functional connections, replace those inactive (or dead) cells, during ageing process. Possibly, continuation of an efficient vestibular function is important for the normal behaviour of the animal.

Introduction

During development and growth, the ganglionic volume and cellular packing density vary greatly, and they do not correspond with each other. On the contrary, in some stages during development, there is an increased ganglionic volume while there is a reduction in the number of cells. In other periods while there is a reduced size of the ganglion, the cellular density is increased. Still in some other stages, there is increase both in ganglionic volume and cellular packing density. More-over, most studies found in a vast review of literature are performed only in certain stages of development or growth which do not give a clear picture of the significance of such variation. Therefore, this study is aimed at analysing the cause of such fluctuation in the whole life cycle of an animal species.

Materials and Methods

The chicks Gallus gallus domesticus, White Leghorn breed were used in this study. Fertilised eggs were incubated. After every 24 hours, it was considered as Embryonic Day 1 (E1), Embryonic Day 2 (E2) etc till hatching (H). Embryos till hatching were removed carefully and fixed in 10 per cent formaldehyde solution at least for two weeks. Larger (older) embryos were cut transversely into suitable smaller pieces and labelled serially for future orientation. The tissues of older embryos (i.e., E15 and onwards till adult) were usually decalcified after fixation. Serial sections of 8 - 10 microns were stained by Cresyl Fast Violet for Nissl granules. Only few selected stages that showed some remarkable changes are described in this work. These include E6, E8, E10, E13, E15, E18, chicks, on the day of hatching (H) and adult (A). In all, three animals in each group, having a total of twenty-four animals were used. Ganglia of both sides in each animal were used for observation.

Every section of the ganglion was observed and drawn. The cells are plotted in a diagram with the help of light microscope having a camera lucida attachment. Different categories of neurones were classified into dark and light neurones according to the difference in the intensity of cytoplasmic stain. Each of these types is again subdivided into various subclasses represented in the diagram by a symbol. Only those cells having a clear nucleus and a nucleolus were counted and measured with the help of an eye- piece graticule. The dimension of every cell was determined by calculating the average of the two measurements: one taken on its long axis at the place where the nucleus and its nucleolus were very clearly observed, and another taken at an angle perpendicular to this long axis. However, the possible error in calculating the size of the cells is considered to be very minimal or negligible. The following categories of cells were classified. Tiny (< 5 microns), very small (6 - 10 microns), small (11 - 15 microns), medium sized (16 - 20 microns), big (21 - 25 microns), very big (26 - 30 microns), large (31 - 35 microns). The categorisation of cells on the basis of size with a uniform difference of 5 microns was initially maintained just for the sake of convenience. However, this proved to be very useful in that, the behavior of cells, especially that of the very-small cells, is very interesting on

the day of hatching (uniformly) in all the ganglia studied. This explains that this particular stage of cellular growth (very-small cell stage) is a critical period during development, indicating a stage of active cell-process-formation (axon-formation) beginning to establish functional connections with the target tissues. This information is also used to interpret the functional significance of the occurrence of light cells during early stages of development.

Results

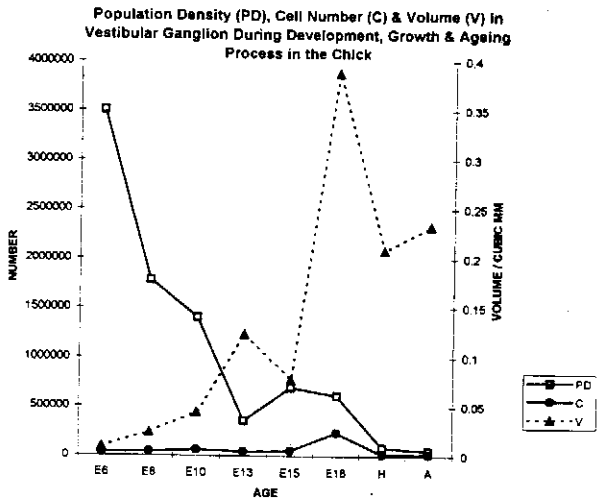
Population density (P. D = number of cells / volume) for certain types of cells is given in parenthesis. When the dark cells alone are present, they are represented just by their numbers and when they are mixed with light ones, D = dark cells, and L = light cells. The most striking changes are described below. The vestibular ganglion could be recognised on E6 while it had a rostro-caudal length of 0.304 mm and a volume of 0.0094 mm^3. The ganglion had 32924 cells (P. D = 3502553). Among these cells, 32916 (99.98 %) were dark type (P. D = 3501702) and 8 (0.02 %) were light ones (P. D = 851). In all, there were 2220 (6.74 %) tiny cells (P. D = 236170), 14803 (44.96 %) very small ones (P. D = 1574787), 15197 (46.16 %) small ones (greatest P. D = 1616702) and 704 (2.14 %) medium sized ones (P. D = 74893). On E8, the ganglion had a length of 0.480 mm and a volume of 0.0240 mm^3, and 42695 cells (P. D = 1778958), and all of these cells were dark type. In all, there were 874 (2.05 %) tiny cells (P. D = 36417), 30870 (72.3 %) very small ones (greatest P. D = 1286250), 10750 (25.18 %) small ones (P. D = 447917) and 201 (0.47 %) medium sized ones (P. D = 8375). On E10, the ganglion had a length of 0.963 mm, a volume of 0.0442 mm^3 and 61936 cells (P. D = 1401267). All of these cells were dark type. In all, there were 10268 (16.58 %) tiny cells (P. D = 232308), 40467 (65.34 %) very small ones (greatest P. D = 915543), 9418 (15.21 %) small ones (P. D = 213077) and 1783 (2.88 %) medium sized ones. On E13, the ganglion had a length of 1.020 mm, a volume of 0.1232 mm^3 and 43894 cells (P. D = 356282). Among these cells, 40267 (91.74 %) were dark type (P. D = 326842) and 3627 (8.26 %) were light ones (P. D = 29440). In all, there were 2674 (6.09 %) tiny cells (P. D = 21705), 27480 (62.61 %) very small ones (greatest P. D = 223052), 9251 (D = 6818 + L = 2433) (21.08 %) small ones (P. D = 75089), 3655 (D = 2665 + L = 990) (8.33 %) medium sized ones (P. D = 29667), 763 (D = 583 + L = 180) (1.74 %) big ones (P. D = 6193) and 71 (D = 47 + L = 24) (0.16 %) very big ones (P. D = 576). On E15, the ganglion had a length of 0.940 mm, a volume of 0.0770 mm^3 and 53439 cells (P. D = 694013). Among these cells, 47465 (88.82 %) were dark type (P. D = 616429) and 5974 (11.18 %) were light ones (P. D = 77584). In all, there were 3449 (6.45 %) tiny cells (P. D = 44792), 31886 (59.67 %) very small ones (greatest P. D = 414104), 10090 (D = 7501 + L = 2589) (18.88 %) small ones (P. D = 131039), 5654 (D = 3195 + L = 2459) (10.58 %) medium sized ones (P. D = 73429), 1609 (D = 953 + L = 656) (3.01 %) big ones (P. D = 20896), and 751 (D = 481 + L = 270) (1.41 %) very big ones (P. D = 9753). On E18, the ganglion had a length of 1.230 mm, a volume of 0.3874 mm^3 and 237191 cells (P. D = 612264). Among these cells, 234877 (99.02 %) were dark type (P. D = 606291) and 2314 (0.98 %) were light ones (P. D = 5973). In all, there were 91241 (38.47 %) tiny cells (P. D = 235521), 100958 (42.56 %) very small type (greatest P. D = 260604), 32555 (D = 31988 + L = 567) (13.73 %) small ones (P. D = 84035), 10248 (D = 8981 + L = 1267) (4.32 %) medium sized ones (P. D = 26453), 1827 (D = 1491 + L = 336) (0.77 %) big ones (P. D = 4716) and 362 (D = 218 + L = 144) (0.15 %) very big ones (P. D = 935). On the day of hatching, the ganglion had a length of 1.200 mm, a volume of 0.2079 mm^3 and 18067 cells (P. D = 86902). Among these cells, 4871 (26.96 %) were dark type (P. D = 23429) and 13196 (73.04 %) were light ones (P. D = 63473). In all, there were 105 (0.58 %) tiny cells (P. D = 505), 4179 (D = 428 + L = 3751) (23.13 %) very small ONES (P. D = 20101), 4987 (D = 924 + L = 4062) (27.6 %) small ones (P. D = 23987), 6640 (D = 2178 + L = 4462) (36.75 %) medium sized (greatest P. D = 31938), 1531 (D = 693 + L = 838) (8.47 %) big ones (P. D = 7364), 498 (D = 425 + L = 73) (2.76 %) very big ones (P. D = 2395) and 127 (D = 118 + L = 9) (0.7 %) large ones (P. D = 611). In the adult situation, the ganglion had a length of 1.290 mm, a volume of 0.2325 mm^3 and 12483 cells (P. D = 53691). Among these cells, 121 (97.64 %) were dark type (P. D = 52426) and 294 (2.3 %) were light ones (P. D = 1265). In all, there were 11 (9.48 %) tiny cells (P. D = 5088), 4935 (39.53 %) very small ones (greatest P. D = 21226), 3800 (D = 3645 + L = 155) (30.44 %) small ones (P. D = 16344), 1961 (D = 1860 + L = 101) (15.71 %) medium sized ones (P. D = 8434), 182 (D = 168 + L = 14) (1.46 %) big ones (P. D = 783), 295 (D = 278 + L = 17) (2.36 %) very big ones (P. D = 1269) and 127 (D = 120 + L = 7) (1.02 %) large ones (P. D = 546).

Discussion

The results in the vestibular ganglion show that there is fluctuation in the ganglionic volume, which does not seem to correspond with the fluctuation in the cell population. The ganglionic volume on E6 is 0.0094 mm^3 that shows gradual increase through E8 and E10 while the cell population also is increasing. The relationship between the size of the cell body, cell number and the volume provides an indication that it is the expansion of the cell processes that dominate in resulting an increased size of the ganglion. This suggests that the ganglionic-volume increase is basically due to the increased volume of neuropil and cell-size. It is also evident from the result

Table 1: Illustrates the total number of dark and light cells in the vestibular ganglion in different age-Groups of Animals in the ontogeny of the chick. (D = Dark Cells, L = Light cells, E = Embryonic age, H = Day of Hatching, A = Adult)

Age	Size										
	Tiny <5u	Very small 6-10u	Small 11-15u	Medium 16-20u	Big 21-25u	Very big 26-30u	Large 31-35u	Very large 36-40u	Glant >40u	Total Number	Grand Total
E6-D	2220	14803	15197	696	0	0	0	0	0	32916	
E6-L	0	0	0	8	0	0	0	0	0	8	32924
E8-D	874	30870	10750	201	0	0	0	0	0	42695	
E8-L	0	0	0	0	0	0	0	0	0	0	42695
E10-D	10268	40467	9418	1783	0	0	0	0	0	91936	
E10-L	0	0	0	0	0	0	0	0	0	0	61936
E13 D	2674	27480	6818	2665	583	47	0	0	0	40267	
E13-I	0	0	2433	990	180	24	0	0	0	3627	43894
E15 D	3449	31886	7501	3195	953	481	0	0	0	47465	
E15 L	0	0	2589	2459	656	270	0	0	0	5974	53439
E18-D	91241	100958	31988	8981	1491	218	0	0	0	234877	
E18 L	0	0	567	1267	336	144	0	0	0	2314	237191
H-D	105	428	924	2178	693	425	118	0	0	4871	
H L	0	3751	4063	4462	838	73	9	0.	0	13196	18067
A D	1183	4935	3645	1860	168	278	120	0	0	12189	
A-L	0	0	155	101	14	17	7	0	0	294	12483



	PD	C	V
E6	3502553	32924	0.0094
E8	1778958	42695	0.024
E10	1401267	61936	0.0442
E13	356282	43894	0.1232
E15	694013	53439	0.077
E18	612264	237191	0.3874
H	86902	18067	0.2079
A	53691	12483	0.2325

PD = Population Density, C = Cell Number, V = Volume
E= Embryonic Day, H= Day of Hatching, A= Adult

Fig. 1: Population density (PD), cell number (C) and volume (V) in vestibular ganglion during development, growth and ageing process in the chick.

some investigators (Hamburger and Levi-Montalcini, 1949; Rubel *et al.*, 1976) that major changes in the cell-size, nuclear size and cell number occur between E13 and the day of hatching in different parts of the central nervous system. The ganglionic-volume increase is minimal during the beginning of development, i.e., up to E10, while as from E13 onwards it shows a quicker and greater increase. However, on E13 the cell population has dropped down. There is a coincidence of reduced number of cells (i.e., degeneration) and the appearance of large number of light cells during this period. This is similar to the suggestions of some of the investigators (Rubel *et al.*, 1976) that most cell death in the brain-stem auditory nuclei occur in the period between E11 and E13. The ganglionic volume is gradually increasing except on two situations: one on E15 while there is a gross reduction in its length and a small decrease in its average cross-sectional diameter and another on the day of hatching while there is a fractional decrease in its length combined with a gross-reduction in its cross-sectional diameter. The ganglionic volume on E15 is reduced while the cell population has increased. However, during the whole embryonic period, the volume reaches its maximum size (0.3874 mm^3) on E18 while the cell population and its density are also the greatest. The volume drops down again to 0.2079 mm^3 on the day of hatching but increases only slightly through further growth to reach 0.2325 mm^3 in the adult situation.

Unlike the condition observed in many other ganglia studied

(Pillay, 1999), the vestibular ganglion has its greatest volume on E18 and not in the adult situation. However, the rostrocaudal length of the ganglion is greatest only in the adult animal. Such behaviour of the vestibular ganglion, having a reduced ganglionic volume in the adult is probably because of their much-reduced functional capacity in the adult due to ageing process.

The appearance of few light cells on E6 for the first time might indicate an active and early establishment of functional connections around this period. A few investigators (Hamburger and Narayanan, 1969), working on the trigeminal ganglion suggested that by the 8th day of incubation at least some of its cells have established peripheral and central connections as indicated by the presence of reflexogenic responses to tactile stimulus of the beak. Still others (Noden, 1980) have found that many trigeminal sensory cells have extensive peripheral projections by the end of the first week of incubation and suggested that these cells might cease dividing very early in embryonic development. Such period coincides with the appearance of light cells in the trigeminal ganglion on E8 (Pillay, 1999). Probably these light cells have formed as a result of their failure to establish a functional connection. Therefore, it is possible to assume that those cells which fail to establish proper central and peripheral connections might become inactive and take a lighter cytoplasmic colour on staining.

This in turn greatly emphasises the importance of the sharp functioning of the vestibular ganglion and vestibular organs during development so as to keep suitable position of the vital bodily organs in relation to gravitational force (which is probably an important factor for normal development and growth of the embryo). The size of cell-population on E6 is 13.87 per cent that shows an increase through E8 to reach 26.11 per cent on E10. This drops down on E13 to 18.51 per cent and increases to 22.53 per cent on E15. Later, there is a sharp increase of cell-population on E18 with a great influx of smaller categories of cells, especially tiny and very small types to gain its highest value of 100 per cent. First increase in the cell-population observed in the ganglion on E10 might be mainly concerned with neural elements (nerve cells and neuroglia), whereas the second increase observed on E18 concerns mainly with phagocytic cells that help remove the unsuccessful neuronal elements. Later these phagocytic cells themselves disappear from the ganglion on the day of hatching. Therefore, the cell population suddenly drops down on the day of hatching while only 7.62 per cent cells remain, with a loss of 92.38 per cent of cells during this short period. Similar finding of enormous number of phagocytic cells at similar stages has been reported by several investigators (Pilar and Landmesser, 1976).

The cell death and degeneration could be one of the factors that regulate the size of the cell population and morphogenesis in the ganglion. Similar function has been attributted by several investigators (Hamburger and Levi-Montalcini, 1949; Saunders, 1966). A few others (Michael *et al.*, 1971) have suggested that the degenerating cells produce hydrolytic enzyme for their own degeneration and digestion. However, the present results do not provide any evidence for this suggestion because the unsuccessful neuronal elements seem to be digested by phagocytic cells which are produced during the last stages (around E18) of embryonic development, most of which disappear befo *the day of hatching. However, their suggestion might* reasonable for the phagocytic cells themselves that appe around E15 - E18 and disappear on the day of hatchin However, if the above suggestion is to be taken for t neuronal elements themselves, as they have suggested, meaning could be attributed for the tremendous producti of the tiny and other smaller classes of cells which appe around E15 - E18 and disappear before the day of hatchi It has been found that about 45 per cent of the total ce observed on E6 fail to survive on the day of hatching. T is similar to earlier findings (Hamburger, 1975) that 40 or more of the neurones that are initially generated fail survive to maturity. There is a further reduction in t number of cells to leave just 5.26 per cent in the adult t seems to be the lowest value in the whole ontogeny. T is attributed to functional reduction due to ageing proce

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