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Cellular Death as a Regulative Mechanism in the Control of Orderly Structure and Function in the Geniculate Ganglion During Development and Aging: An Investigation in the Chick

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The research work was conducted to study the behaviour of dark and light neurones in the geniculate ganglion during development and growth in chick, The dark and light cells are differentiated in each group according to the intensity of cytoplasmic stain. The tiny cells are newly formed cells that are considered as very young and the most active ones, and they are always dark. Cell death (apoptosis) is most prominent and common among the small and medium sized ones. Probably it is during these stages of cell-growth during development, the peripheral and central processes (of axons) begin to grow from the cell body and attempt to get established in their projection fields. If they succeed in their attempt, they continue to function and remain as dark cells, but if they fail they lose their activity, tend to die and disappear, and change into light coloured cell on staining. The light cells have appeared among the very-small cells for the first time just on the day of hatching. This could signify the possible attempt to eliminate the growing cells since they no longer needed to replace larger categories of cells which have already developed functional connections at this stage while the animal is prepared for an independent living. Usually the light cells are reduced very much in number on E18 indicating probably a stage of faster and active removal of inactive and dead cells by the tremendously increased phagocytic cells. The light cell stage becomes clearly observable only when the phagocytic process is slow and becomes prominent at a time when some of the important connections are being actively established. It is assumed that the appearance of light cells might be indicative to the onset of establishment of functional connections of the neurones.

Key words: Geniculate ganglion, apoptosis, dark and light neurones, development and ageing

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Introduction

Dual cytology of neurones (Dark and Light cell-types, based on staining properties) has been documented in vertebrates (Meyer *et al.*, 1973) including primates (Carmel and Stein, 1969). Neurones of these two populations has been the subject of considerable speculations. Dual embryonic origin (of epidermal placode and neural crest) (Hamburger, 1961), as fixation artefacts (Cammermeyer, 1962) different sensory functions (Noden, 1980) different histogenetic characteristics (Meyer *et al.*, 1973) etc. have been offered as different hypothesis. Difference in chemical constituents in these two types of neurones in sensory ganglia has been found in reptiles (Kishida *et al.*, 1982) rodents (Silbermann and Finkelbrand, 1978) and other mammals (Peach, 1972).

There is no report of such study involving the whole life cycle of any one animal so as to infer a conclusive significance of this dual cytology of neurones. All the works have been done either on the adult animals or during certain stages of development or growth. Therefore, it is thought useful to study this in the geniculate ganglion during embryonic development through adult in the chick so as to form a conclusive significance and hypothesis for the occurrence of these two types of neurones.

Materials and Methods

The chicks *Gallus gallus domesticus*, White Leghorn breed were used in this study. Fertilised eggs were incubated and after every 24 hours, it was considered as Embryonic Day I (E1), Embryonic Day 2 (E2) etc. till hatching (H). Developmental stages till hatching were removed carefully and fixed in 10% formaldehyde solution at least for two weeks. Larger (older) embryos were cut transversely into suitable smaller pieces and labeled 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 μ thick were stained by Cresyl Fast Violet for Nissl granules. Only a few selected stages which showed some remarkable changes are described. These include E6, E8, E10, E13, E15, E18 chick 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 and therefore, 48 ganglia in all were used for observations.

Every section of the ganglion was observed drawn and the cells were plotted in diagram with the help of light microscope having a camera lucida attachment. Different size-categories of neurones were classified into Dark and Light ones according to the difference in the intensity of cytoplasmic stain. The following categories of cells were classified. Tiny (< 5 μ), very small (6 - 10 μ), small (11 - 15 μ), medium sized (16-20 μ), big (21 - 25 μ), very big (26 - 30 μ), large (31-35 μ), very large (36 - 40 μ), giant (41 - 45 μ), gigantic (46 - 50 μ).

Results

During development and growth of the animal, the geniculate ganglion showed great difference in different age groups of animals and in different areas of the same ganglion. When dark neurones alone are present in the ganglion, they are represented just by numbers, however, when they are mixed with light neurones D= dark neurones, and L= light neurones. The ganglion could be clearly recognised on E6 while it had 3428 cells and all of them were dark type. In all, there were 95 (2.77%) tiny cells, 1232 (35.94%) very small ones, 1815 (52.95%) small ones, and 286 (8.34 %) medium sized ones. On E8, the ganglion had 6705 cells, all of them were dark type. In all, there were 1952 (29.11 %) tiny cells, 3111 (46.4%) very small ones, 1542 (23 %) small ones and 100 (1.49%) medium sized ones. On E10, the ganglion had 1361 cells and all of them were dark type. In all, there were 81 (5.95%) tiny cells, 160 (11.76%) very small type, 677 (49.74%) small ones, 343 (25.2%) medium sized ones and 100 (7.35%) big ones. On E13, the ganglion had 4764 cells of which 4252 (89.25%) were dark type and 512 (10.75 %) were light ones. In all, there were 78 (1.64%) tiny cells, 1841 (38.64%) very small type, 774 (D= 693 + L= 81) (16.25%) small ones, 964 (D= 807 + L= 157) (20.24%) medium sized ones, 1017 (D= 765 + L= 252) (21.35%) big ones,

61 (D= 45 + L= 16) (1.28%) very big ones and 29 (D= 23 + L= 6) (0.61%) large ones. On E15, the ganglion had 2869 cells of which 1442 (50.26%) were dark type and 1427 (49.74%) were light ones. In all, there were 98 (3.42 %) tiny cells, 935 (32.59 %) very small type, 515 (D=150+ L=365) (17.95%) small ones, 908 (D= 147 + L= 761) (31.65%) medium sized ones, 317 (D= 74 + L= 243) (11.05%) big ones, and 96 (D= 38 + L= 58) (3.35%) very big ones. On E18, the ganglion had 17592 cells of which 16543 (94.04%) were dark type, and 1049 (5.96%) were light ones. In all, there were 8092 (46%) tiny cells, 5533 (31.45 %) very small type, 228 (D= 1971 + L= 317) (13.01%) small ones, 1559 (D= 920 + L+ 639) (8.86%) medium sized ones, 106 (D=20+L= 86) (0.6%) big ones and 14 (D= 7 + L= 7) (0.08%) very big ones.

On the day of hatching, the ganglion had 2093 cells of which 1112 (53.13 %) were dark type and 981 (46.87%) were light ones. In all, there were 20 (0.96 %) tiny cells, 183 (D=59+L= 124) (8.74%) very small type, 328 (D= 69 + L= 259) (15.67%) small ones, 603 (D= 289 + L= 314) (28.81%) medium sized ones, 480 (D= 301 + L= 179) (22.93%) big ones, 473 (D= 368 + L= 105) (22.6%) very big ones, 2 (0.1 %) large ones, 1 very large ones and 3 (0.15%) giant dark type of cells.

In the adult situation, the ganglion had 1021 cells of which 904 (88.54 %) were dark type and 117 (11.46%) were light ones. In all, there were 15 (1.47 %) tiny cells, 7 (0.69%) very small type, 36 (D= 22 + L= 14) (3.53%) small ones, 96 (D= 84 + L= 12) (9.4 %) medium sized ones, 22 (D=13+L= 9) (2.15 %) big ones, 212 (D= 194 + L= 18) (20.76%) very big ones, 198 (D= 175 + L= 23) (19.39%) large ones, 349 (D= 315 + L= 34) (34.18%) very large ones, 2 (D= 1 + L= 1) (negligible) giant ones, and 84 (D=78 + L= 6) (8.23%) gigantic type of cells.

Discussion

Only dark cells are observed up to E10, the light cells begin to appear on E13 and continue through the whole ontogeny of the chick. The tiny cells are found to be always dark. The dark and light cells are dispersed at random through out the ganglion as from E13 through the rest of the ontogeny unlike the observations of other investigators (Noden, 1978) who found such random distribution only in mature (from 18th day of incubation to adult situation) ganglia i.e. from shortly before hatching up to adult, and not in the younger stages. The very small cells were found to be dark through the whole embryonic period till E18. The light cells have appeared for the first time, among this very small type on the day of hatching but have disappeared in the adult situation. This might imply that even though the very-small cells appear to keep themselves active till the day of hatching and be ready to replace the dead cells which would occur because of several adverse factors, cell death and degeneration begin among this category also as from the day of hatching. To assume that no necessity to establish new functional projections after the day of hatching because all of them might have been complete by

Table 1: lustrates the total number of dark and light cells in the geniculate ganglion in different age-groups of animals in the ontogeny of the chick

SIZE AGE	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	Giant >40u	Total Number	Grand Total
E6-D	95	1232	1815	286	0	0	0	0	0	3428	
E6-L	0	0	0	0	0	0	0	0	0	0	3428
E8-D	1952	3111	1542	100	0	0	0	0	0	6705	
E8-L	0	0	0	0	0	0	0	0	0	0	6705
E10-D	81	160	677	343	100	0	0	0	0	1361	
E10-L	0	0	0	0	0	0	0	0	0	0	1361
E13-D	78	1841	693	807	765	45	23	0	0	4252	
E13-L	0	0	81	157	252	16	6	0	0	512	4764
E15-D	98	935	150	147	74	38	0	0	0	1442	
E15-L	0	0	365	761	243	58	0	0	0	1427	2869
E18-D	8092	5533	1971	920	20	7	0	0	0	16543	
E18-L	0	0	317	639	86	7	0	0	0	1049	17592
H-D	20	59	69	289	301	368	2	1	3	1112	
H-L	0	124	259	314	179	105	0	0	0	981	2093
A-D	15	7	22	84	13	194	175	315	79	904	
A-L	0	0	14	12	9	18	23	34	7	117	1021

D= Dark cells, L= Light cells, E= Embryonic day, H= Day of hatching, A= Adult

this time while the animal is ready to lead an independent living. No need for further growth and maturation of these very small cells any more. It is therefore possible to expect that cell death begins even among these very-small cells as from the day of hatching, in order to prevent their further growth which seems unnecessary.

The results in the geniculate ganglion on E6 show that there are 3428 cells all of which were dark. On E8 the cells have increased in number to 6705 and the ganglion showed an active cellular proliferation as indicated by the increased number of tiny cells and an active cellular growth and maturation process as evidenced by the presence of an increased number of very small cells. The reduced number of small as well as medium sized cells is suggestive of cellular death, degeneration and loss taking place during this period, and that this degeneration process concerns mainly with larger classes of cells and these cells are the critical stages in development while an attempt to develop proper projection is taking place. When this attempt fails, the cells could not grow and therefore could not live, become inactive or die, and on staining, they change into light coloured cells (so-called light-cells).

This resembles the suggestion of some of the earlier investigators (Cowan, 1973) that the cell degeneration is influenced by peripheral field of the innervation and that the cell death can be influenced by the target cells of a neurone population.

E10 appears to be critical stage in which there is a great cellular loss (having just 1361 cells) with a loss of about 80% from that observed on E8 and this is observed among all categories of cells including the tiny ones. This suggests that there is a great reduction or probably a complete stoppage of cellular proliferation, as well as an increased cellular death and degeneration during this stage. It may be suggested that the cellular death, degeneration, removal and loss are taking place very fast so that the light-cell stage is not observable. It is assumed that E10 is a critical stage in the development while an attempt to establish proper projection among the neuronal elements. When this attempt fails, the cells could not live and therefore, die and this process are more accentuated around E10 - E15 with certain amount of fluctuation (cellular increase) on E13.

Total number of cells has increased on E13 (to 4764 cells), the reduced number of 78 tiny cells suggests a reduction or stoppage of proliferative activity. Very-small, small, medium sized and big cells have increased in number and the new appearance of very-big and large types of cells indicates that there is a continued growth and maturation while proliferative activity has reduced. The appearance of light cells for the first time on E13 (even though small in number = 512 light cells) is suggestive that cellular death and degeneration, it indicate that the continued attempt to establish functional innervation (around E10), so that the unsuccessful cells change into light coloured cells which become observable on E13. The appearance of light cells in trigeminal ganglion (Pillay, 1999) as early as E8 coincides with the experimental findings (Noden, 1980) that some of the trigeminal ganglion cells have established peripheral and central connections as evidenced by the presence of reflexogenic responses to tactile stimulus of the beak and the connections of geniculate ganglion cells are established around E10 - E13.

E15, it may be noticed that even though the total number of cells has reduced (to 2869 cells), the proportion of dark (1442 cells = 50.26%) and light (1427 cells = 49.74%) cells is almost equal, having an increased proportion of light cells compared to the earlier stage E13. This indicates a higher incidence of cell death and degeneration during E15. Total disappearance and reduction of large cells during this stage might imply that the cell death is most prominent among larger classes. The death and degeneration occur after going through all their attempts to establish functional connections while at the same time, they continue to grow and mature. When they fail to establish functional connections they are removed by phagocytosis.

E18 there is a great increase in the cell-population (with 17592 cells) having a greatly increased number of 16543 dark cells and a reduced number of 1049 light ones. The presence of great number of a tiny cells and an increased number of larger classes might indicate an accelerated rate of cellular proliferation, growth and maturation. The reduced number of light cells as well as the reduction in the number of big (to 106 cells) and very-big type (to 14 cells) might mean that they have failed to develop functional connections and have died. A comparative analysis of the on E18 and those observed on the day of hatching shows that there is a great cellular loss between these two periods resulting in the presence of 2093 cells on the day of hatching. This huge cellular loss mainly concerns with the large number of tiny, very-small, small and even medium sized ones. This indicates that the multiplicity of tiny cells observed on E18 concerns mainly with the phagocytic cells, or cells which have gained the capacity to remove the unsuccessful neuronal elements. Similar findings are reported by (Wang-Chu and Oppenheim, 1978).

The results are similar to the CNS (Levi-Montalcini, 1949) that changes in cell size, nuclear (neurone-group) size and cell

number occur between E13 and the day of hatching and the ganglionic volume increases at the end of cellular death which begins around E13 - E17, immediately after a great cellular loss is observed on E10. The " nuclear " size of these investigators may be correlated with the " ganglion " size in the present study since corresponding and parallel changes have been observed (Rubel *et al*., 1976) in the brain stem auditory nuclei and their peripheral ganglionic projections in the chicken. It is apparent that the cellular death and degeneration could be one of the factors that regulate the size of cellular population in this ganglion (Saunders, 1966) who point out the role of this phenomenon in morphogenesis.

The comparative analysis of the results on E18 and on the day of hatching, it can be noticed that the number of cells is greatly reduced (on the day of hatching) to just 2093 cells having 1112 dark and 981 light ones. The rate of cellular proliferation has also greatly reduced or stopped on the day of hatching as evidenced by the presence of reduced number of just 20 tiny cells. However, even though other classes of cells very small, small and medium sized ones have reduced in number, the bigger classes, the big and very-big types have increased, along with the new appearance of large, very-large and giant types.

In the adult situation again, a great cellular loss is noticed having a total of just 1021 cells containing 904 dark type and 117 light ones. The loss of cells is spread through all categories of cells and an enormous loss of cells in the adult might represent a functional reduction due to increasing age. The gigantic cells have appeared for the first time in adult situation.

In many situations through the whole ontogeny, new cells are being formed or added by active proliferation of tiny cells with some interrupted periods of rest or reduced proliferation while the total number of cells actually is not declining. This The supports the view (Hamburger, 1958) that there are degenerating cells at a time when the number of cells was not actually declining and that new cells must have been entering. Similarly in Xenopus (Hughes, 1961) it has been calculated that the number of degenerating cells was greatly in excess that required to account for the decline in cell numbers and therefore, concluded that the turn-over was taking place in the developing ventral horn. In this study, there were 3428 cells generated on E6 and 2093 cells were left on the day of hatching which amounts to 39% cellular loss. Hamburger, (1975) that 40% or more of the neurones that are initially generated fail to survive to maturity. In the adult situation, only 1021 cells are left which amounts to a loss of 70% of cells from that initially generated (i.e., from that observed on E6). However, this great loss is attributed to the changes occurring as a result of ageing process (or functional reduction).

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