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Neurobehavioral Morphology of Superior Cervical Ganglion during Neurogenesis and Aging Process

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This study concerns with neurobehavioural morphology during the development and growth of the superior cervical ganglion. Dark and light cell types, their categorization and significance during development are described. On the evidence available, the dark cells are considered as active ones and the light cells as those which have failed to establish functional projections, inactive, dying, dead or degenerating ones. It is reasonable to assume that the optimum number of functional neurons should have been formed by the day of hatching while the animal is prepared and ready for an independent living. The ganglionic volume is the greatest on E18 during the whole embryonic period. The increased ganglionic volume on E18 might be related to a combined effect of tissue reaction resulting from the toxic elements of dead cells along with the phagocytic activity. However, the ganglion shows its greatest volume in the adult situation in the whole ontogeny. The cell-space available for every cell is the greatest in the adult situation while the ganglionic volume is greatly increased and the number of cells is greatly reduced. The establishment of functional connections ends very late in this ganglion in contrast to other ganglia studied. The present results also confirm that the functional activity of this ganglion remains active even in the adult situation in contrast to many other ganglia where the number of neurons and possibly their activity are greatly reduced later in the adult. On further analysis of the present results with that of other ganglia studied it is observed that the interaction and functional coordination of this ganglion with that of nodose and vestibular ganglia seem to be important for proper development and growth of all organs during prenatal development.

Key words: Superior cervical ganglion neurogenesis, neurobehavioural morphlogy, aging, *Gallus domesticus*

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INTRODUCTION

Great many changes have been described during the development of neural structures in several animal species (Peach 1972; Meyer *et al*., 1973; Pillay, 1999, 2000a, b) including primates (Kerr, 1967; Carmel and Stein, 1969). This includes the studies relating to the size and density of neural elements (Ptacek and Fagan-Duban, 1974; Pillay, 1999, 2000a, b), dual embryonic origin (Hamburger, 1961), difference in sensory functions (Noden, 1980; Spassova, 1982), different histogenetic characteristics (Meyer *et al*., 1973), distribution of cytoplasmic organelles (Carmel and Stein, 1969) and neuronal death (Wang-Chu and Oppenheim, 1978; Pillay, 2001, 2003).

However, most workers have confined their studies to a few selected stages during embryonic development or postnatal growth. There is no systematic study performed covering the whole life of any one animal species thereby proper interpretation of the developmental and growth changes could be formulated. Therefore, this study is aimed at analyzing the structural organization occurring in the superior cervical ganglion during embryonic development, growth and adult situation so as to formulate and confirm the significance of these changes.

MATERIALS AND METHODS

The chicks Gallus domesticus, White Leghorn breed were used in this study. Fertilised eggs were incubated. After every 24 h, 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% 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 10 microns were stained by Cresyl Fast Violet for Nissl granules. Only a 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 (i.e., 48 ganglia in all) were used for observation.

Every section of the ganglion was observed and drawn. The cells were plotted in a diagram with the help of a light microscope having a camera lucida attachment. Different categories of neurones were classified into dark and light neurones based on the difference in the intensity of cytoplasmic stain (Fig. 1). 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 measurement taken on its long axis at the place where the nucleus and its nucleolus were clearly observed and another taken at an angle perpendicular to this. 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) etc.

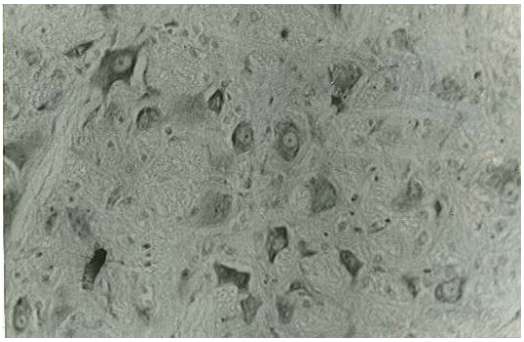

Fig. 1: Dark and Light neurons in the ganglion as indicated by staining characteristics

RESULTS

The superior cervical ganglion could be recognized on E6 while it had a rostro-caudal length of 0.560 mm and a volume of 0.0083 mm^3 and had 14489 cells (Population Density = PD = 1745663) all of which were dark type. In all, there were 687 (4.74%) tiny cells (PD = 82771), 5986 (41.31%) very small type (PD = 721205), 7250 (50.04%) small ones (greatest PD = 873494) and 566 (3.9%) medium sized ones (PD = 68193) (Table 1).

During the following days, several changes in the distribution and density of different types of cells, in the composition and volume of the ganglion etc took place. The ganglion showed great difference in the different age groups of animals and in different areas in the same ganglion. The most striking changes are as follows.

On E8, the ganglion had a length of 0.600 mm, a volume of 0.0325 mm^3 and had 30832 cells (PD = 948677) all of which were dark type. In all, there were 5230 (16.96%) tiny cells (PD = 160923), 12194 (39.55%) very small type (PD = 375200), 13190 (42.78%) small ones (greatest PD = 405846) and 218 (0.71%) medium sized

Table 1: Total number of dark and light cells in the superior cervical ganglion in different age groups of animals in the ontogeny of the chick

	Size										
Age	Tiny <5 u	Very small 6-10 u	Small 11-15 u	Medium 16-20 u	Big 21-25 u	Very big 26-30 u	Large 31-35 u	Very large 36-40 u	Giant >40 u	Total No.	Grand Total
E6-D	687	5986	7250	566	0	0	0	0	000	14489	
E6-L	0	0	0	0	0	0	0	0	0	0	14489
E8-D	5230	12194	13190	218	0	0	0	0	0	30832	
E8-L	0	0	0	0	0	0	0	0	0	0	30832
E10-D	10832	31144	3929	1776	0	0	0	0	0	47681	
E10-L	0	0	0	0	0	0	0	0	0	0	47681
E13-D	347	22999	12004	3972	0	0	0	0	0	39322	
E13-L	0	0	0	0	0	0	0	0	0	0	39322
E15-D	1292	59282	11798	2602	0	0	0	0	0	74974	
E15-L	0	0	0	0	0	0	0	0	0	0	74794
E18-D	38420	42647	44252	1912	0	0	0	0	0	127231	
E18-L	0	0	323	168	0	0	0	0	0	491	127722
H-D	675	9404	7086	1007	0	0	0	0	0	18172	
H-L	0	28679	8154	239	0	0	0	0	0	37072	55244
A-D	475	8228	8365	1057	146	0	0	0	0	18271	
A-L	0	11798	4116	155	34	0	0	0	0	16103	34374

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

ones (PD = 6708). On E10, the ganglion had a length of 0.657 mm, a volume of 0.0387 mm^3 and had 47681 cells (PD = 1232067) all of which were dark type. In all, there were 10832 (22.72%) tiny cells (PD = 279897), 31144 (65.32%) very small ones (greatest PD = 804754), 3929 (8.24%) small ones (PD = 101525) and 1776 (3.72%) medium sized ones (PD = 45891). On E13, the ganglion had a length of 0.580 mm, a volume of 0.0582 mm^3 and had 39322 cells (PD = 675635) all of which were dark type. In all, there were 347 (0.88%) tiny cells (PD = 5962), 22999 (58.49%) very small ones (greatest PD = 395172), 12004 (30.53%) small ones (PD = 206254) and 3972 (10.1%) medium sized ones (PD = 68247). On E15, the ganglion had a length of 0.690 mm, a volume of 0.1075 mm^3 and had 74974 cells (PD = 697431) all of which were dark type. In all, there were 1292 (1.72%) tiny cells (PD = 12018), 59282 (79.01%) very small ones (greatest PD = 551460), 11798 (15.72%) small ones (PD = 109748) and 2602 (3.47%) medium sized ones (PD = 24205). On E18, the ganglion had a length of 0.720 mm, a volume of 0.1963 mm^3 and had 127722 cells (PD = 650647). Among these cells, 127231 (99.62%) were dark type (PD = 648146) and 491 (0.38%) were light ones (PD = 2501). In all, there were 38420 (30.08%) tiny cells (PD = 195722), 42647 (33.39%) very small ones (PD = 217254), 44575 (D = 44252 + L = 323) (34.9%) small ones (greatest PD = 227075) and 2080 (D = 1912 + L = 168) (1.63%) medium sized ones (PD = 10596).

On the day of hatching, the ganglion had a length of 0.810 mm, a volume of 0.1166 mm^3 and had 55244 cells (PD = 473791). Among these cells, 18172 (32.89%) were dark type (PD = 155849) and 37072 (67.11%) were light ones (PD = 317942). In all, there were 675 (1.22%) tiny cells (PD = 5789), 38083 (D = 9404 + L = 28679) (68.94%) very small ones (greatest PD = 326612), 15240 (D = 7086 + L = 8154) (27.59%) small ones (PD = 130703) and 1246 (D = 1007 + L = 239) (2.26%) medium sized ones (PD = 10686). In some of the sections, the light cells were comparatively more numerous in their dorsal part while the dark cells were observed to be more in their ventral part.

In the adult situation, the ganglion had a length of 1.430 mm, a volume of 0.4928 mm^3 and had 34374 cells (PD = 69752). Among these cells, 18271 (53.15%) were dark type (PD = 37076) and 16103 (46.85%) were light ones (PD = 32676). In all, there were 475 (1.38%) tiny cells (PD = 964), 20026 (D = 8228 + L = 11798) (58.26%) very small ones (greatest PD = 40637), 12481 (D = 8365 + L = 4116) (36.31%) small ones (PD = 25326), 1212 (D = 1057 + L = 155) (3.53%) medium sized ones (PD = 2460) and 180 (D = 146 + L = 34) (0.52%) big ones (PD = 365).

DISCUSSION

The categorisation of cells on the basis of size with a uniform difference of 5 microns was initially maintained for the sake of convenience. However, this proved to be useful in that, the behaviour of cells especially that of very-small ones, is 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) indicating the 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.

The greatest cell population, considered as 100%, is observed on E18, on the basis of which the percentage of

cellular population for other age groups of animals is calculated. The cell population on E6 is 11.34% that seems to be the lowest value in the whole ontogeny. This value increases through E8 to reach 37.33% on E10 which drops down to 30.79% on E13. Then the cell-number shows a sharp increase through E15 to reach its highest value of 100% on E18. Such increase mainly concerns with the tiny and very small type of cells. Then the population-size drops down to 43.25% on the day of hatching with a loss of 56.75% cells. On further growth of the animal, there is again a reduction to leave just 26.91% of cells in the adult situation. The first increase in the cellular population observed on E10 might be concerned mainly with neural elements (neurons and neuroglia) while the second raise observed on E15 - E18 possibly concerns mainly with phagocytic cells. These phagocytic cells would help remove the unsuccessful neuronal elements that fail to establish functional projections (Pillay, 2000a, b, 2001) and therefore, these cells die. Later the phagocytic cells themselves disappear from the ganglion.

All the cells observed on E6 are dark type. This condition continues to be the same through E8, E10, E13 and E15. The light cells have been observed for the first time on E18 that continue to be a constant feature on the day of hatching and in the adult situation. The tiny cells are always found to be dark type. The very small cells are also dark through the whole embryonic stages till E18. However, on the day of hatching some of these cells are found to be light ones. This condition remains to be the same even in the adult situation. Since most of the ganglionic cells are of dark type through the whole embryonic stages till E 18, it is assumed that majority of the ganglionic cells are very actively involved in establishing functional connections till the very end of fetal period. However, it may also be assumed that the replacement of inactive cells are done very fast before the light cell stage begin to appear in the vicinity of the ganglion. It is possible that this condition is related to the active life of the bird immediately on hatching.

Moreover, the developmental behaviour of the superior cervical ganglion has some relationship with the observations in nodose and vestibular ganglia as well (Pillay 1999, 2000a, b). Possibly, these three ganglia play a role in the balanced and coordinated physiological activity and importance of these three ganglia in performing a faster functional activity of the animal immediately on hatching including faster walking. Possibly this coordinated physiological development of all these three ganglia (nodose, sympathetic, as well as vestibular ganglia) are also essential to provide a balanced development and growth of all other organs in the body. It may be useful and interesting to verify whether the developmental behaviour of these ganglia in the human, primates and other species of mammals are comparable with those of birds.

The results in the superior cervical ganglion show that its rostrocaudal length is gradually increasing at every stage of development through the whole ontogeny till adulthood except on E13 where there is rather a slight reduction. The ganglionic volume is continuously increasing through the whole embryonic period till E18 with a gradual increase in length and in average cross-sectional dimensions except on E13 while the ganglion shows an increased volume because of its increased cross-sectional diameter, in spite of its reduced length. On the other hand, the ganglionic volume reduces on the day of hatching while there is a great loss of its average cross-sectional diameter, in spite of its increased length. The increase in ganglionic volume might be due to several factors such as the recruitment of additional migratory cells moving away from the neural crest/placode and to the proliferation of cells which have reached their final position in the ganglion, considerable growth of the cell body of individual elements, the increase in the number of satellite cells and development of interstitial spaces and blood vessels (Pillay, 1999, 2001). However, even though there is a fluctuation in the ganglionic volume and in the number of cells within the ganglion at different stages of development, the relationship between the size of the cell body, cell number and the ganglionic volume provides an indication that it is the expansion of the cellular processes which dominates in resulting an increased size of the ganglion. Even though there is some sort of fluctuation in the ganglionic volume from time to time, the ganglionic size-increase is minimal during the beginning of development, i.e., up to E10 while as from E13 onwards the ganglion shows a quicker and greater increase in volume. The present results in the superior cervical ganglion show that the ganglionic volume increases gradually till E13. However, when the cell death comes to an end (i.e., around E13) the number of cells has greatly reduced and that there is a rapid increase in the ganglionic size during the short period between E13 and the day of hatching. Late phase of ganglionic growth in the avian system in the present study might be an example of the regulative control that the afferent activity can exert on neuronal ontogeny. The cell loss has essentially ended (by E13) by the time the ganglion begins (around E13) to show its greatest ganglionic expansion so as to reach its maximum size on E18.

It is also evident from the results of Levi-Montalcini (1949) and Rubel *et al.* (1976) that major changes in cell size, nuclear size and cell number occur between E13 and the day of hatching in different cell groups in the central nervous system. The nuclear size of these investigators

may be correlated with the ganglionic size in the present investigations because Rubel *et al.* (1976) found corresponding and parallel changes taking place both in the brain-stem auditory nuclei and their peripheral ganglionic projections. The results in the nodose ganglion in the present series of study (Pillay, 1999) also show similar tendency and the ganglionic volume increases greatly just after the cell death comes to an end around E13 so as to reach its maximum size on E18. This observation is similar to that described by Rubel *et al.* (1976) in the brain stem auditory nuclei in the chicken. It may be assumed from the descriptions of Rubel *et al.* (1976) that increase in ganglionic volume commences at the end of the period of cell death which might suggest that in the present study the cell death comes to an end around E13 while the moderately increased ganglionic volume shows some degree of fluctuation with a slight decrease on E13, even though the process of further degeneration and removal of dead cells might continue even later. In the embryonic period there is a rapid increase in ganglionic size during a short period especially between E15 and the day of hatching which is similar to the observation of Rubel *et al.* (1976) in the brain stem auditory nuclei which they correlated with the corresponding and parallel changes taking place in the peripheral ganglionic projections as explained above. Similarly synchronous growth of the functionally related cell groups has also been described in the visual system of golden hamster (Ptacek and Fagan-Duban, 1974) where cell density decreases and cell size increases in both the visual cortex and superior colliculus in a parallel fashion. Even in mammalian visual system (Globus, 1975) similar phenomena have been repeatedly shown to be dependent upon the integrity of visual stimulation. It is also noticed that there appears to be an initial small increase in the ganglionic volume (up to E10) then there is a fall during the period of rapid cell loss (on E13).

On E8 there is an increase in the number of tiny, very small and small cells in the ganglion even though the number of medium sized cells is reduced from that observed on E6. This indicates that even in the absence of light cells in the ganglion, there is a cellular loss especially among the medium sized ones. This could mean that the increased number of cells includes another category of cells capable of phagocytosis. The phagocytosis is so fast that the inactive or dead cells are immediately removed from the vicinity of the ganglion before the light-cell stage becomes observable. And, at the same time, the newly formed (tiny) cells continue to grow into larger classes, possibly by the development of functional connections in the target tissue. This would mean that the unsuccessful and weak cells are quickly being removed from the ganglion in order to be replaced by another group of actively growing cells so as to take over their function without delay. Those cells that succeed in this process of establishing functional projection would survive and that others would die. Cell death and degeneration could possibly be influenced by the peripheral field of innervation or in other words by the target tissue of a neuron population. It might also be assumed that the cells might undergo exhaustion due to some defects in the inherent capacity (endogenous factor) or in the microenvironment (exogenous factor) at any stage of their development and growth. This resembles the suggestion of Hamburger and Levi-Montalcini (1949), Hamburger (1958), Cowan and Wenger (1967) and Cowan (1973) that cell degeneration is probably influenced by the peripheral field of innervation and that cell death can be influenced by the target cells of a neuron population.

There is a great increase in the total number of cells on E10 along with an increase in almost all categories of cells except the small ones that has a reduced number (from 13190 of E8 to 3929 cells on E10). These results show that the greatest cellular loss takes place within the small (on E10) as well as the medium sized (on E8) ones. This suggests that these cellular groups represent critical stages of cellular growth, at which these cells possibly develop axonal and dendritic processes in an attempt to establish proper functional connections. However, when these cells fail in their attempt they might become inactive, die and disappear from the ganglion. At the same time, some of these cells have escaped cellular death and have grown into medium sized ones. It is assumed that these cells are successful in establishing a functional projection onto the target tissues. The increased number of tiny and very small types of cells indicates an active proliferation of new cells as well as their continued growth. However, during all these developmental stages, the largest class of cells remains to be just medium sized ones which imply that this is the maximum size to which the neurons of the superior cervical ganglion can normally reach during development.

The total number of cells has reduced to 39322 on E13 in which the tiny and very small type of cells has been greatly lost which would directly mean a reduced rate of proliferation and growth. However, the increased number of small (to 12004 cells) and medium sized cells (to 3972 cells) might mean further growth of the very small type. Therefore, it is possible to note an alternate period of proliferation and growth of these cells with an intermediate period of some type of rest or inactivity in the ganglion. From the results observed in the majority of ganglia in the present series of investigation (Pillay, 1999, 2000a, b, 2001, 2003) it is possible to assume that the small and/or medium sized cells represent critical stages while the cells are attempting to establish functional

connections. During early stages of development, i.e., as from E8-E13, phagocytosis is also an active process that is taking place too fast that the inactive or dead cells are being removed immediately before the light cell-stage becomes observable (Pillay, 1999, 2003). It might also be assumed that the period of accelerated degeneration is the period of active establishment of functional connections of the ganglion cells. Similar loss of cells during E11-E13 and increase on E15-E18 in trigeminal ganglion were also observed by Eric *et al.* (1999) by their immunochemical tracing techniques. The cellular death and degeneration could be an important factor that regulates the size of cellular population in the ganglion.

This is some-what similar to the observation of Rubel *et al.* (1976) about cell death which they observed among the cells in the brain stem auditory nuclei where most cell death occur in the period between E11 and E13. It has been shown by Yip and Johnston (1984) that survival of mature neurons of the dorsal root ganglion in new born rats is partially dependent on the availability of Nerve Growth Factor (NGF) transported from the CNS via the dorsal root fibers. This also supports the suggestion that those cells which have established proper functional projections will receive sufficient NGF from the CNS and survive and that others will die. However, it is possible to assume that even after establishment of functional projection if these fibers are unable to transport the NGF or if the NGF available for transport through the fibers is inadequate to keep the optimum amount these cells might die as well. This might imply that the cellular death can occur at any stage of their growth, probably even after a connection is established. This could be possible that when the peripheral projection obtained by these cells are not in the proper field or when there is a defect in the micro-environment which probably has developed later secondarily either in the projection field or in their passage, these connections might lose their function. Therefore, these cells might be destined to die by an adverse stimulus (such as inadequate supply of NGF) coming from the CNS as being responsible to promote nerve growth in the dorsal root ganglion in the new born rat as it has been explained by Yip and Johnston (1984).

The results on E15 show that there is an active proliferation, growth and maturation process as evidenced by the presence of an increased number of tiny cells and a greater number of very small ones. The reduced number of small and medium sized cells is suggestive of a definite occurrence of cellular death and degeneration among these groups of cells. However, even at this stage, the absence of light-cells while there is cellular loss is suggestive of a much faster cellular death, degeneration and removal of inactive or dead cells before the light-cell stage becomes observable. These results indicate that all cellular activities including proliferation, growth, maturation, death, degeneration and removal are taking place at the same time among different types of cells in the ganglion.

On E15 the tiny and very small cells in the ganglion show an enormously increased number. This is suggestive of a greater rate of proliferation, growth and maturation of cells in the ganglion. However, the largest class of cells continues to be the medium sized ones (16-20 mm^3) only, similar to that observed on E13. This seems similar to the suggestion that adverse factors are present either within the cells themselves (endogenous factors) or in the micro-environment (exogenous factors) as being responsible for cell death (Pillay, 2001, 2003). It may also be possible that the cells which fail to establish functional projection probably begin to die as a result of inadequate supply of stimulus such as NGF which is responsible for further growth and survival (Yip and Johnston, 1984) which is normally needed to promote nerve growth. Even though this view suits very well with the present results, the proliferation of new cells, their growth and maturation continue fast during this period, probably in order to replace such unsuccessful neuronal population which could not establish themselves as functional neurons. However, in spite of such reduction in the small and medium sized cells the total number of cells has actually increased to 74974 cells on E15 which is mainly due to the increased number of tiny and very small ones. It may be thought that such increased number of cells might also include larger proportion of phagocytic cells in addition to neuronal elements since the ganglion is preparing for phagocytic activity as explained later with the behavior of the ganglion on E18 and establishment of neuronal projections should have been completed by this stage while the animal is almost ready to be hatched to lead an independent active life.

Hamburger and Narayanan (1969) working on the trigeminal ganglion have suggested that by the 8th day of incubation at least some of the ganglion cells have established peripheral and central connections as indicated by the presence of reflexogenic responses to tactile stimulus of the beak. Noden (1980) has found that many trigeminal sensory cells have extensive peripheral projections by the end of first week of incubation and suggested that these cells must cease dividing very early in embryonic development. It is during this period (E8) in the present series of investigation in the trigeminal ganglion (Pillay, 1999) that the light cells have made their first appearance as well. This coincidence of the experimental evidences given by the above workers regarding the establishment of extensive peripheral and central connections by 8th day of incubation and the appearance of light cells for the first time in the trigeminal

ganglion during the same developmental stage (E8) might indirectly but clearly suggest that these light cells are formed as a result of the failure in making functional connections. It is assumed that these cells might become ultimately inactive, lose their function, die, degenerate and disappear and that these are the cells that take a lighter color on staining and that this light-cell stage represents early stages of cellular inactivity or degeneration. This resembles the suggestion of Hamburger and Levi-Montalcini (1949), Hamburger (1958), Cowan and Wenger (1967) and Cowan (1973) that cell degeneration is probably influenced by the peripheral field of innervation and that cell death can be influenced by the target cells of a neuron population. However, it might also be assumed that cells might undergo exhaustion due to some defects in the inherent capacity (endogenous factor) within the cells or in the micro-environment (exogenous factor) at any stage of development and growth even though the establishment of proper connection might play an important role in this process (Pillay, 2003). However, the light cell stage does not occur in the superior cervical ganglion possibly because of the faster removal of inactive cells from the vicinity of the ganglion.

On E18, there are greatly increased number of dark cells (127231 cells) and a few light ones (491 cells) while there is also an active proliferation and cellular growth as represented by an increased number of tiny, very small and small cells. However, the reduced number of medium sized cells represents cellular death and degeneration. The presence of light cells among the small and medium sized categories indicates that cell death and degeneration are taking place predominantly among these groups of cells. Even though, a degree of speculation might be given for an active removal of dead cells (as it has been observed in earlier stages of development), it is during this period (E18) a definite degeneration process is clearly represented by the presence of some light cells. However, the largest class of cells continues to be the medium sized ones till E18. Possibly, this is an inherent nature of the superior cervical ganglion that the termination of active establishment of functional connections is too much delayed unlike other ganglia studied (Pillay, 1999, 2000a, b, 2001, 2003).

A comparative analysis of different types of cells observed on E18 and those observed on the day of hatching shows that there is a great cellular loss during this period. This loss concerns mainly with large proportion of tiny, small and medium sized ones whereas the very small cells have slightly reduced in number. Since most of the cells on the day of hatching belong to the very small and small categories, it might also be assumed that these categories of cells are the major functional groups during this period. Similar assumption is developed even in the adult situation. Therefore, these classes of cells (very small and small types) might be considered as important functional groups in this ganglion. However, in the adult situation, a few big cells (146 D cells + 34 L cells = 180 cells) have also formed. The active influx of tiny cells and, their further growth on E18 possibly concern mainly with another category of cells with a capacity for phagocytosis in order to help remove the inactive or dead neuronal elements which have failed to establish functional connections (Pillay, 2001, 2003).

The presence of enormous number of phagocytic cells at similar stages (E18) has been reported by several investigators. Levi-Montalcini (1950) suggested that phagocytic cells derive from macrophages and serve to digest neuronal debris during cell death. Tennyson (1970) says that satellite cells act as phagocytes to remove the cellular debris in embryonic ganglia. O'Connor and Wyttenbach (1974) and Pilar and Landmesser (1976) have reported that phagocytosis is accomplished entirely by glial cells. Wang and Oppenheim (1978) have observed blood-borne mononuclear leukocytes acting as phagocytes. It is observed from the present results that major changes in cell size, cell number and ganglion size take place between E10 and the day of hatching. This behavior is almost similar to the report in the CNS by Levi-Montalcini (1949) and Rubel *et al.* (1976) that major changes in cell size, nuclear size (neuron-group) and cell number occur between E13 and the day of hatching and that the increase of ganglionic volume commences at the end of the period of cell death which begins around E13-E18 as explained earlier. The cellular death and degeneration could be one of the factors that regulate the size of cell population in the ganglion as has been suggested by Hamburger and Levi-Montalcini (1949), Glucksmann (1951) and Saunders (1966) in their studies in which they attributed that the cell death was influenced by the peripheral field of innervation and thus point out the role of this phenomenon in morphogenesis. Michaels *et al.* (1971) suggested that degenerating cells produce hydrolytic enzyme for their own degeneration and digestion. However, the present results have no evidence for this suggestion because the phagocytic cells produced during this period should have performed their active function for a short period and disappeared by the day of hatching. However, this suggestion might be reasonable for the phagocytic cells themselves which appear around E15-E18 and probably are responsible to remove the remnants of the degenerating neuronal elements. If the above suggestion is to be taken for the neuron themselves, then no explanation or meaning could be attributed for the tremendous production and flooding of the tiny cells which appear around E15-E18 and disappear by the day of hatching. All these cellular and

morphologic changes have been confirmed in the present series of investigation in different ganglia (Pillay, 1999, 2000a, b, 2001, 2003).

On the day of hatching, a great reduction in the total number of cells to 55244 cells (from 127722 cells on E18) is associated with the appearance of a larger proportion of light cells among very small, small and medium sized ones. It is also observed that these light cells are predominantly observed among the very small ones. These results indicate greater cell loss among smaller classes, viz very small and small ones. The occurrence of greater number of light cells in the ganglion on the day of hatching suggests either a greater cellular death and degeneration, or a reduced phagocytosis. The great reduction in the number of cells on the day of hatching might be due to faster and active cellular death and degeneration along with an increased phagocytosis and removal of these cells from the vicinity of the ganglion.

In the adult situation, there is a greater cellular loss (with a total of 34374 cells in all) with a greater loss among light cells (from 37072 light cells on hatching to 16103 cells in the adult) whereas the number of dark cells remains almost the same (18172 cells on hatching and 16103 in the adult). Cellular loss among light-cell group at many circumstances adds to the assumption that these light cells represent a group of inactive, dead or degenerating cells. The appearance of big cells (146 D + 34 L = 180 cells) for the first time in the adult situation represents further growth and maturation of some of these cells during post-hatching period. The tiny cells have only slightly reduced in the adult (to 475 cells) in comparison to that observed on the day of hatching (675 cells). The major loss is observed among the very small cells, that too among the light-cell group. However, there is a small reduction in the number of dark cells as well. The very small cells have reduced in the adult situation. The loss among the small cells also concerns mainly with the light-cell group. However, the small dark cells have slightly increased in number in the adult situation, possibly due to growth and maturation of some of the very small cells. Similar loss in the number of light cells and increase in the number of dark cells also appear among the medium sized category. The growth and maturation of these surviving cells result in the formation of big cells for the first time in the adult ganglion. But the number of dark cells remains almost the same even in the adult situation, as that observed on the day of hatching. It is possible that the functional connections of these neurons should have been fully established on the day of hatching and continues to remain the same even in the adult. This could confirm that the functional activity of this ganglion continues to be active even in the adult situation in comparison to many other ganglia studied in the present series of investigation (Pillay, 1999, 2000a, b, 2001, 2003).

In many situations through the whole ontogeny, new cells are being formed or added by an active proliferation of tiny cells with some interrupted periods of rest or reduced proliferation while the number of total cells is actually not declining. This indirectly but clearly, supports the view of earlier investigators (Hamburger, 1958; Pillay, 2001, 2003) 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) calculated that the number of degenerating cells was greatly in excess of that required to account for the decline in cell number and therefore concluded that the turn over was taking place in the developing ventral horn.

There are more than one period of active proliferation and active degeneration which are indicated by the sharp fluctuations (raise and decline) in the number of cells in different stages of development and growth. This is similar to the observation of Carr and Simpson (1978) that beyond day 8 of incubation there is a second period of degeneration affecting dorsomedial neuron population in both brachial and thoracic ganglia in their study. Cowan and Wenger (1967), Rogers and Cowan (1973) and Hamburger (1975) have found that 40% or more of the neurons which are initially generated fail to survive to maturity. This trend has been observed during the development of various cranial nerve ganglia as well (Pillay, 1999, 2000a, b, 2001, 2003).

On comparing the results observed on the day of hatching, in the adult situation there is a reduction in the number of light cells only while the dark cells appear to remain almost the same. This would suggest that the autonomic function by sympathetic system tends to remain active even in the adult situation as that on the day of hatching. However, it is reasonable to assume that the optimum number of functional neurons should have been present when the new animal is ready for an independent living, i.e., on the day of hatching with normal development of all organs and systems in the body, leaving a chance for the cells for further growth and functional maturation later in life. However, in the superior cervical ganglion, since the loss of cells is observed mainly among the light-cell group (which is supposed to be already inactive or dead), it might be assumed that the functional loss by ageing process is minimal in this autonomic ganglion.

In the present study, the dark and light cells are dispersed at random throughout the ganglion as from E18 onwards through the rest of the ontogeny. The behaviour of these cells in the superior cervical ganglion is strikingly different from that observed in other cranial nerve ganglia studied in the present series of investigation (Pillay, 1999, 2000a, b, 2001, 2003) where the light cells began to appear even from the early stages of development, the period

varying mostly within E6-E13. Here in the superior cervical ganglion only dark cells are found up to E15 and the light cells begin to appear for the first time on E18 and continue to be present through the whole ontogeny of the chick. The tiny cells are always dark. The very small cells are also dark through the whole embryonic period till E18. Later on the day of hatching however, the light cells have appeared among the very small cells also and continue to be present even in the adult situation. This suggests that even though the very small cells appear to keep themselves to be an active group before the day of hatching (i.e., during embryonic development) and be ready to replace the dead cells which might occur because of several adverse factors, cell death and degeneration begin among these cells (as indicated by the occurrence of light cells) as from the day of hatching. It may be assumed that normally there is no need for the establishment of new functional projections after the day of hatching because all these connections should have been already completed by this time while the animal is ready to lead an independent living. Therefore, there is no need for further growth and maturation of this smaller class of cells and cell death begins even among them as from the day of hatching, in order to prevent further growth of these cells which seems unnecessary.

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