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STEREOLOGICAL ANALYSIS OF THE INFERIOR OLIVARY NUCLEUS IN THE DEVELOPING HUMAN BRAIN

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ABSTRACT

The stereological analysis of the inferior olivary nucleus was performed on 25 human fetal brains aged from 8th to 31st gestation week (GW) and on the brain of a newborn. The fetal brains were obtained on hysterectomy due to myoma uteri or after spontaneous abortion because of cervical incopetency.

The brains were fixed in 10% formalin solution, embedded in celloidin and paraffin and cut in frontal plane in 6, 15 and 30 /um and stained with cresyl violet. The stage of maturation of the nerve cells was established on the basis of the degree of basophilia, metachromatic charcteristic of the nucleoli and colouring of the cytoplasm and the occurrence of the Nissl bodies. In all of the investigated stages the diameter of the nerve cell nuclei was determined by the eye-piece graticule. Stereological analysis was performed by using Weibel`s M 42 test system. The results obtained indicate that the nerve cells of the second stage of maturation were observable up to the 16.5th GW, , and the cells of the third stage from 17.5th to 22nd week GW. Parallely with cells of the third stages of maturation in the 22nd GW the cells of the fourth stage were conspicuous until birth. The volume of the nerve cell nuclei was increasing until birth. A rise in average volumes was statistically significant ($p<0.001$). The value of the numerical density of the nerve cell nuclei was decreasing in the course of development. The decrease of the numerical density of the nerve cell nuclei was statistically significant up to the 16.5th GW. Between the 16.5th and 19.5th GW the decrease of the numerical density of the nerve cell nuclei was statistically insignificant Since 19.5th GW until the birth the decrease of the numerical density of the nerve cell nuclei was, again statistically significant ($p<0.001$).

KEY WORDS: inferior olivary nucleus, man, development, stereology.

INTRODUCTION

The inferior olivary nucleus (IO) occurs only in mammals, and consists of a dorsal and ventral lamina. With ascent of their evolutionary scale the inferior olive undergoes a progressive increase in relative size, reaching its greatest development in man. This increase is said to be parallel the development of the pontine nuclei and the cerebellar hemisphere (Marsden and Rowland, 1965). After pontine nuclei the inferior olive is the most important precerebellar relay nuclei. The inferior olive is considered as the only one or the most important source of the climbing fibers (Armstrong, 1974, Batini et al., 1976, Courville and Farco-Cantin, 1978). The human climbing fibers are distributed in the internal granular layer, within narrow, and long vertical territories

which are transverse to the long axis of the folium (Marin-Padilla, 1985). The human climbing fibers are characterized by multineuronal target and by establishing contacts with small groups of Purkinje cells rather than with isolated neurons. A climbing fiber sends collaterals to several cerebellar folia as it passes by them, further corroborating their multineuronal targets. They also send collaterals to the internal granular layer, some of which form pericellular nests around the body of the Purkinje, Lugaro and Golgi cells (Marin-Padilla, 1985). According to the trophic theory the climbing fiber input is essential to maintain the Purkinje cells in normal functional state. The tonic theory considered that one of the most peculiar properties of the climbing fiber input is to fire at rather low rate (Strata, 1984). Some investigations suggest that each olivary cell corresponds to a "piece of output" which could take many forms; it might be a fine digit or limb movement. Sometimes a "piece of output" is called elemental movements, and each olivary cell is supposed to correspond to one elemental movement. It is supposed that olivary dictionary of elemental movements is complete and every possible action can be represented as an ordered pattern of elemental movements each of which has a special olivary cell. It means that every action has a defining representation as a sequence of firing patterns in the olive. Having in mind the previous facts it is obvious that there are a lot of papers which deal with IO in a number of animal species and in man (Kooy, 1917, Scheibel and Scheibel, 1955, Strata, 1984, Gudović et al., 1988).There is a small number of papers dealing with the determination of the number of the inferior olivary nerve cells in man (Moatamed, 1966, Escobar et al., 1968). Cytomorphometric and stereological analysis of the IO in man during development has not been performed yet. The aim of this investigation was to shed more light on IO during prenatal development by using stereological methodology.

MATERIAL AND METHODS

For this study 25 human fetal brains aged from the 8th to the 31st GW and one newborn were used. The fetuses were obtained after spontaneous abortion because of cervical incompetence of the uterus (11.5-31 GW), and the youngest fetuses on hysterectomy due to the myoma uteri (8, 8-9, and 9.5 GW). The neonate died after respiratory distress. The fetuses were free of diseases involving brain. The fetal ages were determined after Olivier-Peneau (Kostović, 1979). The fetal brains were fixed in 8% and 10% formalin solution for four weeks, embedded in paraffin and celloidin and cut in frontal plane at 6, 15, and 30 μm. The sections were stained with cresyl violet. The histological analysis was done at serial sections under light microscope at different magnification. The stage of maturation of nerve cells was established on the basis of the degree of basophilia, metachromatic characteristics of the nucleoli and colouring of the cytoplasm and the occurrence of the Nissl bodies (Rakić, 1968). For the stereological analysis every 5th section was used. In each section 10 test fields were counted, 5 on the left and 5 on the right side. Totally 80 test fields in each stage were counted. To determine the diameters of nerve cell nuclei the eye-piece graticule was used. The value obtained was multiplied by $4/\pi$ in order to calculate the true diameter, while it was established that there was an obvious linear relationship between the radius (R) of sphere and its mean profile size , and therefore this correction was necessary (Elias and Hyde, 1983). For the stereological analysis the light microscope was used (occ. 10; obj. 100). The nerve cell nuclei were counted at one level of a thick slice using the multipurpose test system M42.

The numerical density of the nerve cell nuclei (N_V), in this case, was calculated by formula (Pajer and Kališnik, 1984):

$$N_V = \frac{N_A}{DF + \overline{D}} \qquad (1)$$

where DF was the subjective depth of focus (Pajer and Kališnik, 1986), NA the number of nerve cell nuclei per unit test area and $\overline{D}$ the mean value of diameters. For calculation PC (XT) was used. The results were statistically evaluated using Student's t-test. The results were graphically presented.

This paper is the last in the series of the papers which deal with the developmental changes of the cerebellum and related structures. The results obtained provide the basis for the determination of the appropriate mathematical models of these structures during development and therefore it was decided to apply the given counting methods, instead of more sophisticated ones.

RESULTS

The cells of the IO appear in the 8th GW and correspond to the second stage of maturation. These cells were present up to the 16.5th GW. In later stages (17.5th to 22nd PW) the cells of the third stage of maturation were noticed. Parallel with the cells of the third stage of maturation in the 22nd GW the cells of the fourth stage were conspicuous until birth. The maturation of the nerve cells was accompanied with their nuclear augmentation (Fig. 1) which was statistically significant ($p<0.001$) up to the birth. The mean values of numerical density of the nerve cell nuclei was decreasing during development. The decrease of the numerical density of the nerve cell nuclei was statistically significant ($p<0.001$) up to the 16.5th GW. Between 16.5th and 19.5th GW the decrease of numerical density of the nerve cell nuclei was statistically insignificant. Since 19.5th GW up to the birth the decrease of the numerical density of the nerve cell nuclei was, again, statistically significant ($p<0.001$) - Fig. 2.

DISCUSSION

It is generally accepted that the neuroblasts forming IO originate from rhombic lip (Sidman and Rakić, 1982). By the end of the XIX century and the beginning of the XX several authors studied its development in man (Hiss, 1889, Essick, 1907, citated after Kappers, 1967, Kooy 1917). After that period the development of the IO in man has not been done. Since 1980 in our laboratory the IO became the object of our investigation (Gudović et al., 1984, Gudović et al., 1988). from different standpoints. The present study was conducted to examine the quantitative or stereological development of the inferior olivary nucleus after 8th GW.

In humans, the number of the nerve cells in the adult IO was calculated by several authors (Moatamed, 1966, Escobar et al., 1968). Their measuring methods were so different from on another that it is difficult to compare ours with their reported data. Our results indicate that numerical density of the nerve cell nuclei was decreasing during development. The loss of the nerve cells during development could be explained by the reasons stated below. First the choice of fixative and embedding media must be careful. For our investigations the formalin as a fixative and paraffin as embedding medium were employed. It is well known that the shrinkage after fixation of the brain in formalin amounts to about 50% in volume (Haug 1972). In our case while the fetal material was used it is understood that the shrinkage was higher in younger fetuses than in the older ones. The second reason is the stage of cell maturation. Namely, it was mentioned that the cells of the second stage of maturation were observable up to the 16.5th GW. The cells of the second stage of maturation were characterized by large, pale nucleus with prominent nucleolus and a very poor chromatin substance. The cytoplasm was still invisible because the granular endoplasmic reticulum has not been sufficiently morphologically and functionally differentiated (Rakić, 1968). On the basis of this fact we may conclude that in the period between the 8th and the 16.5th GW both neuroblasts and spongioblasts were counted together. In other words the numerical density of the nerve cell nuclei in this period was presented as a sum of their numerical densities. In later stages this was not the case, because

these two groups of the cells were easily distinguishable. Exactly, the cells of the third stage of maturation (from 17.5th to 22nd GW) were characterized by pale sickle-shaped cytoplasm. As a result of further development and maturation of the cells, particularly the endoplasmic reticulum the cells of the fourth stage of development occurred. These cells had a large, pale nucleus with prominent nucleolus and a light blue cytoplasm, having Nissl bodies differing in fineness, entirely enveloped the nucleus (Rakić 1968). Besides these facts which may be a cause of decrease of the numerical density, it is well known that during development progressive and regressive phenomena exist (Hamburger and Levi-Montalcini, 1949, Cowan et al., 1984, Carlson et al.,

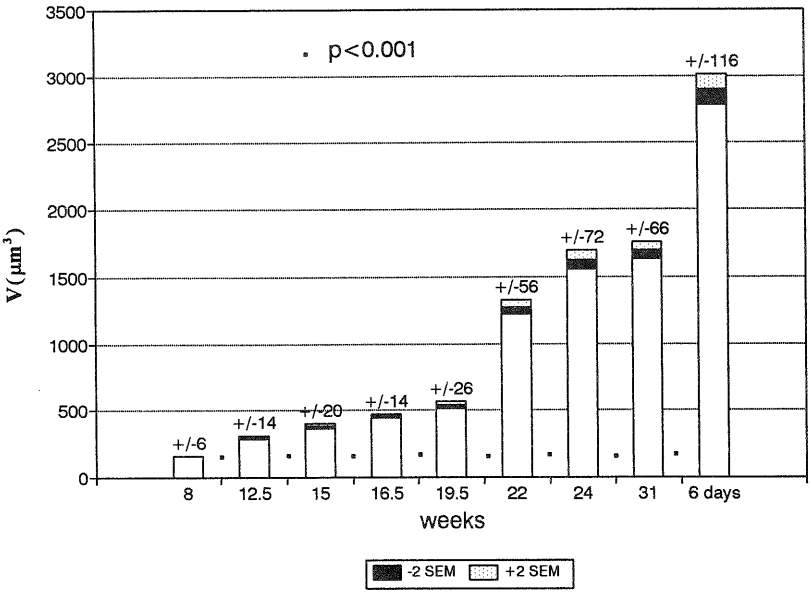

Fig. 1. Average nuclear volumes of IO nerve cells in μm³.

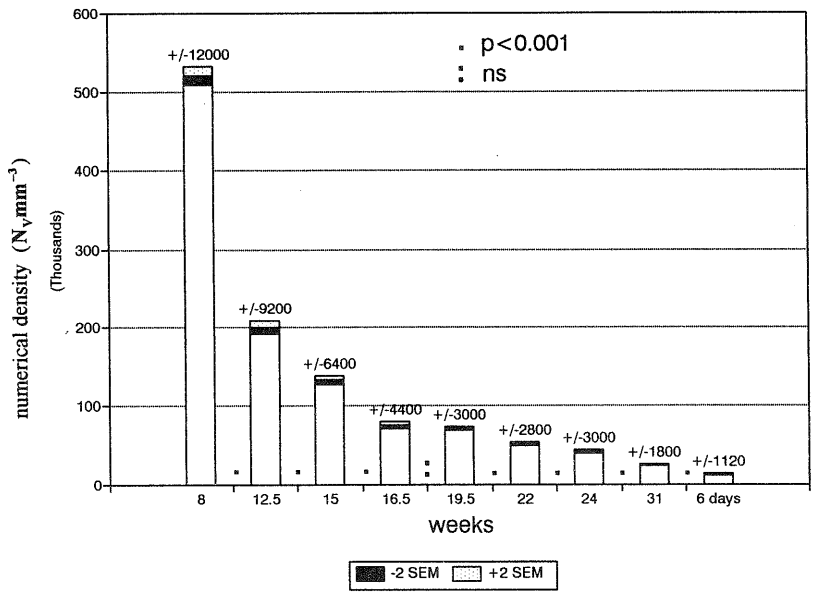

Fig. 2. Numerical density of the nerve cell nuclei (Nv +/- 2SEM) of the IO in the developing human brain.

1988). In human embrios the process of proliferation and migration of the nerve cells lasted up to the 12-14th GW (Rakić and Sidman, 1970), although some findings indicate that this process may last up to the 24th GW (Herschkovitz, 1988) or even longer (Carlson et al, 1988). After period of proliferation and migration, the period of reduction of the nerve cells take place in practically all nerve structure. Carlson et al., (1988) pointed out that reduction of the nerve cells amounts to 20-50%. According to the findings of Oppenheim (1985) the cell number was reduced more then 50%. Our results show a cell number decrease more pronounced. Namely, the numerical density of the nerve cell nuclei in a newborn baby amounted to 2.40% of the numerical density in the 8th GW. Those findings indirectly supported the concept of the target-related cell death (Hamburger 1975, Katz and Lasek , 1978). Practically the nature of this process is still unknown. Some authors consider neuronal competition as an important event in this process (Purves, 1980). Others suggest that there is a linear relationship between the projection fields and a rate of cell death (Herschkovitz, 1988). Some of them supposed that the regressive phenomena is theoretically linked to sex differences, temperamental traits and perceptual motor coordination (Carslon et al., 1988). Oppenheim (1985) explains the cell death by error of motoneuron projection. In this case it is necessary to emphasize that the human climbing fibers arrive at the Purkinje cell plate of the cerebellar hemispheres by the end of the 28th GW, establishing a paraganglionic plexus of immature fibres before they established recognizable contacts with the Purkinje cells. By the end of the 31st GW all Purkinje cells of the cerebellar hemispheres have pericellular nests around their bodies (Marin-Padilla, 1985). Therefore, it could be supposed, that only the cells able to make an adequate contacts with other cells in time would remain, forming a corresponding functional system. The wrong or functionally useless contacts would be rejected and resulted as the cell death. The reduction of the nerve cell to about 50% between 31st GW and a 6 day old newborn could be explained by previous facts.

Comparing the present with our previous results it is possible to conclude that during development of the IO three period could be distinguished. By the end of the first period (8th-16.5th GW) the both laminae of the IO were formed and were smooth area (Gudović et al., 1984). The cells corresponded to the second stages of maturation. Each week showed an increase of the nuclei. A rise in average volumes was statistically significant ($p<0.001$). The numerical density of the nerve cell nuclei by the end of this period amounted to 14.15% of original values (8th GW). The second period (17.5th GW to the 21st GW) corresponded to the cells of the third stage of gestation. Both laminae showed primarily wrinkles (Gudović et al., 1984,1988). A high statistical significance of the increase of the volumes of nuclei was found ($p<0.001$). By the end of this period (19.5th GW) the numerical density of the nerve cell nuclei amounted to 13.58% of the original values (8th GW). The third period (22nd up to the birth) corresponded to the cells of the fourth stage of maturation. Both laminae showed an enhancement of the secondary wrinkles and augmentation of the nuclei ($p< 0.001$). By the end of this period the numerical density of the nerve cell nuclei amounted to 2.36% of the original values (8th GW).

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