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POSTNATAL NEUROGENESIS IN THE FELINE CEREBELLUM: A STRUCTURAL/FUNCTIONAL INVESTIGATION

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Abstract. The postnatal development of the feline cerebellum was analyzed and correlated with the development of balance and locomotor patterns of behavior. Three major experimental approaches were undertaken to analyze this development: (i) packing densities and linear measurements of the nodulus and uvula lobes of the archicerebellum; (ii) qualitative analysis of changes in cell composition and morphology; and (iii) behavioral observations of the development of locomotor activity. The histological analysis indicated that the external granular layers (EGL) attained its maximum thickness at 15 days followed by a rapid dissolution from this point on; concomitant with this the internal granular layers (IGL) slowly increased from birth to 60 days. Behaviorally the results showed that the 1st postnatal month was taken up with the acquisition of locomotor behavior while the 2nd postnatal month was concerned with the perfection of this behavior. Possible correlations between postnatal neurogenesis and locomotor development were discussed within the framework of brain plasticity.

INTRODUCTION

Earlier studies using thymidine — H^3 autoradiography revealed the presence of a large population of postnatally proliferating neurons in many brain regions in a number of mammalian species (5, 6).

One well known site of postnatal neurogenesis is the external granular layer of the cerebellum, indeed this was suggested nearly a century ago by Obersteiner (11). The mode of proliferation, migration and differentiation of the cells forming this layer have been traced in mice (10), in rats (6) and in dogs (12), and the results of these autoradiographic and histological investigations have conclusively demonstrated that the external granular layer gives rise to the microneurons (basket, stellate and granule cells) of the cerebellum.

The cerebellum as a structure is responsible for the co-ordination and modulation of input and output for motor responses at the reflex, autonomic and conscious levels (7, 8, 16). Within this structure, it is the function of the microneurons to modulate and regulate the interaction among afferent, efferent and higher-order macroneurons (Purkinje and Golgi I cells). An example of such activity is the excitatory and inhibitory activity of the granule cells. These cells when excited by incoming mossy fibers have a direct excitatory influence on cerebellar output by means of the axodendritic parallel fiber connections with Purkinje cells and indirectly influence cerebellar output by way of the parallel fiber connections with the dendrites of the converging and diverging basket cell system which affects Purkinje cell bodies in an inhibitory manner (8).

The postnatal origin and subsequent modulatory role of microneurons prompted Altman to suggest that these may be the plastic elements of the central nervous system, that is, they "control some function or functions which appear or mature after birth" (6, p. 465). In this context he noted the intimate relationship that exists between the duration of postnatal cerebellar neurogenesis in different species and the degree and temporal course of the development of motor coordination in those species (2). Developmental investigations with cats and rats carried out by Tilney and Casamajor (14), Windle (15) and Tilney (13) found that, indeed, balance and locomotor behavior patterns do develop according to a regular sequence of events during the earliest neonatal period.

The absence of a detailed investigation into the dissolution of the external granular layer and concomitant formation of the internal granular layer in kittens and a desire to correlate these morphological changes with the development of specific balance and locomotor behavior patterns prompted this investigation.

MATERIALS AND METHODS

The litters from four domestic cats obtained from registered animal dealers totalling 15 kittens, male and female, were used as subjects. From birth to 60 days postnatally at 5 day intervals (Table I) the kittens were sacrificed by cardiac perfusion with a 10% neutral formalin solution. Removed brains were further fixed in formalin then dehydrated in a graded series of alcohols and embedded in paraplast. Moving caudal to rostral through the cerebellum a series of coronal sections at 10 μ were cut. From these every 15th section was preserved and stained with Einarson's modification of gallocyanin-chromalum.

Ten or six sections (rostral in the nodulus and uvula) from each age group were selected for microscopic evaluation; this evaluation was limited to portions of the flocculonodular lobe, namely the nodulus and the uvula, those portions of the archicerebellum involved in vestibular functioning. Because of the marked developmental changes in the structural appearance of the cerebellum, landmarks in the medulla were used as reference points and every attempt was made to select, from age to age, the same folia always cut in the same manner. Linear measurements and cell counts for both the external and internal granular layers (EGL and IGL) were taken at a microscopic enlargement of 400 ×. For an explanation as to just how the areas were selected and controlled and how many measurements were used, refer to Fig. 1. The crosses indicate the points on the nodulus and uvula lobules of the cerebellar vermis where the cell counts and the linear measurements were taken. The size of the area for cell counts was 0.025 mm^2. For the qualitative data, attention was focused on changes in cell composition and changes in the morphological appearances of cells.

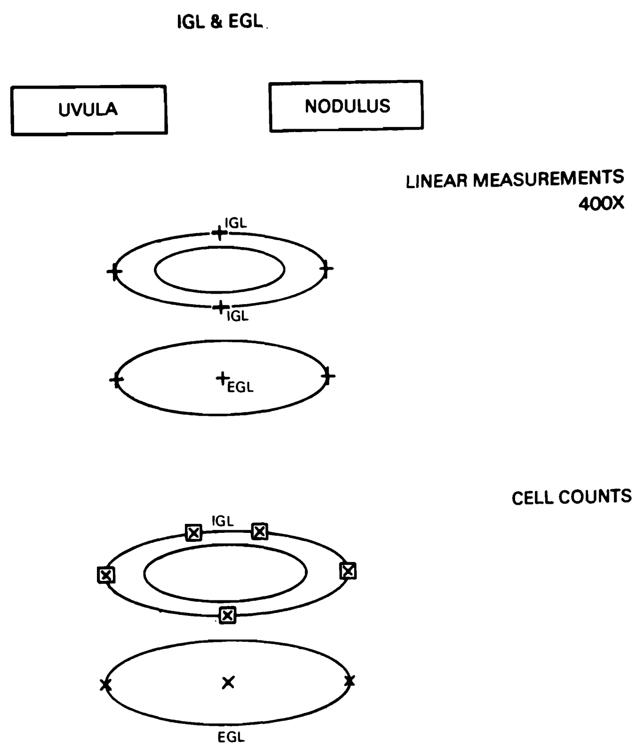

Fig. 1. Schematic indicating the areas using the linear measurements and the cell counts. Abbreviations: IGL, internal granular layers; EGL, external granular layers. Each of these diagrams represents one section. They were then added together and means obtained. For each section in the IGL cell counts, 5 samples × 10 or 6 sections were taken yielding 30 or 50 samples.

Behavioral observations commenced at birth and continued daily for each kitten until it was sacrificed. These observations included the age of the kitten, the date and time of the observation, the balance and/or locomotor patterns observed and a summary of their manner of execution. This procedure was the result of pilot work in which the objective was to determine the reliability of the reactions defined in Table II; in other words, the consistency of the observer in operationally defining the criteria to be employed in the observations was assessed. The reliability coefficient (Kuder–Richardson) obtained for this work was 0.88.

TABLE I
Number of kittens sacrificed in age group

Days (postnatal)	Number of kittens
0	1
5	2
10	2
15	1
20	1
26	1
30	1
35	1
40	1
45	1
50	1
55	1
60	1
	Total 15

RESULTS

Quantitative data

The results of the total mean packing density and the linear measurements for both the external and internal granular layers of the nodulus and uvula lobules of the vermis of the cerebellum are summarized in Fig. 2. A more detailed presentation of this material is to be found in Table III for the external and internal granular layers.

Figure 2 points up clearly the dissolution of the external granular layer and the concomitant formation of the internal granular layer. This phenomenon occurs sharply at 15 days when the EGL has attained its maximum thickness and thereafter begins its rapid dissolution. At the same time the packing density of the IGL, which is 12/0.025 mm^2 at

15 days, nearly doubles to 18/0.025 mm^2 at 60 days postnatally. The linear measurements bear out this occurrence, and the functions are similar in slope to the results of the packing density for both the EGL and the IGL.

TABLE II
Definitions of balance and locomotor patterns of behavior

Postural reaction	The kitten adjusts its position to lie on its belly
Crawl-approach	The simultaneous extension of fore/hind paws which drag the body (in contact with the ground) forward
Synergizing reaction	Swaying movements of the head and trunk acquire precision and steadiness
Eye-opening reaction	Eyes formerly closed now are open. The eye ball is visible to the observer
Eye-head turning reaction	The kitten follows moving objects across the visual field with eyes and rotates its head in a horizontal plane
Anti-gravity reaction	The head and body of the kitten are maintained erect over the four legs and supported by the four paws touching the ground
Sitting reaction	The head and upper body are maintained erect while supported by the collapsed hind legs and the extended fore paws touching the ground
Walking reaction	Simultaneous extension of each pair of fore/hind paws in making forward progress while the head and body are erect over the four legs
Running reaction	The extension of both forepaws and both hind paws in rapid sequence while making forward progress
Climbing reaction	The simultaneous extension of each pair of fore/hind legs which pulls the head and body upward on a surface perpendicular to the ground
Jumping reaction	The hind legs are bent at the knees then forcibly extended, projecting the head and body upward, downward and forward

Table III is presented to show the exact packing density and linear measurements found in each lobule at the various ages examined. For both the EGL and IGL the mean packing densities and linear measurements for each lobule are in close agreement at the various ages. Random recounts of the original areas were taken from two sections for each age group in both the EGL and IGL. The reliability coefficients (Pearson's Product Moment) obtained were 0.85 for the EGL and 0.83 for the IGL.

Qualitative data

The cerebellum of the neonatal kitten (0–10 days) is very small and immature morphologically. The external granular layer is dense and easily distinguishable. The molecular layer is a thin white band containing

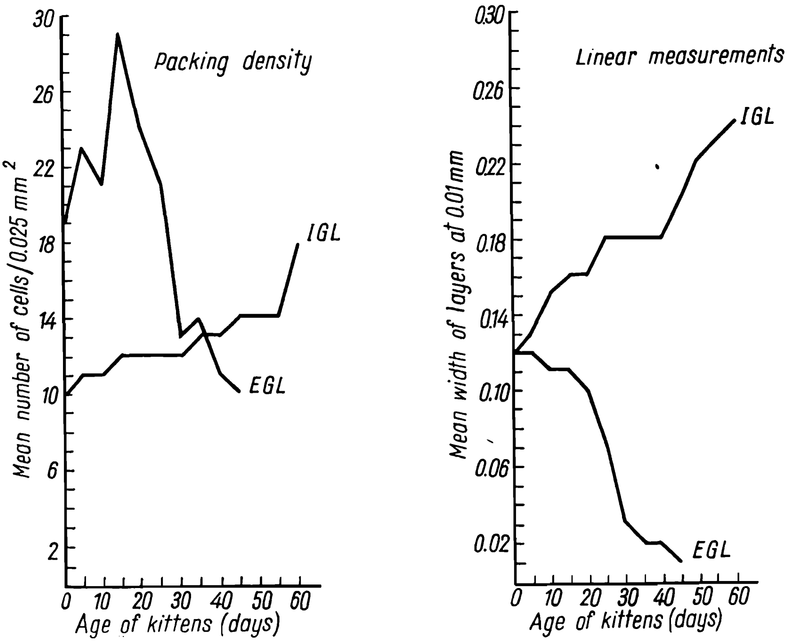

Fig. 2. Packing densities and linear measurements of the external granular layers (EGL) and internal granular layers (IGL) in kittens of various ages.

a few scattered lightly-stained cells and an occasional vertically-oriented spindle shaped cell. The Purkinje cells are small; pale nuclei are interspersed throughout the IGL; and the internal granular layer itself is rudamentary, containing loosely packed granule cells. From 15–26 days the EGL has attained its maximum thickness and begun its rapid dissolution. At 15 days, two zones are easily distinguishable in the EGL. The upper zone is a dense layer of packed round cells several rows thick. Just below this is a zone composed of darkly stained horizontally-oriented, spindle-shaped cells several rows thick. By 26 days, however, there are proportionally more round cells in the upper portion than there are spindle-shaped cells at the border, and the layer itself has been sharply reduced in thickness. The molecular layer during this time slowly continues to increase in size, and more spindle-shaped cells are present. Presumably, these are the indifferent cells moving towards the IGL. The Purkinje cells have now formed into a single row just above the IGL and are easily recognized. The IGL, while increasing slowly in width, is still composed of loosely packed granule cells. From 30–40 days the EGL changes in composition and is considerably reduced in size. At 35 days proportionally more horizontal, spindle-shaped cells are visible just below the now thin band of round cells, and by 40 days the layer is reduced in size to 3–4 cell rows in thickness. The molecular layer of the 30 day old kitten contains many spindle-shaped, vertically oriented cells, and by 40 days these cells actually outnumber the lightly stained cells present in the layer. The internal granular layer increases in width and by 40 days has begun to become densely packed with granule cells. From 45–60 days the EGL remains a vestige composed mainly of spindle-shaped cells. Numerous spindle-shaped cells continue to be visible in the molecular layer up to 60 days at which time it is composed chiefly of lightly stained cells with only a few spindle-shaped cells visible at the very bottom of the layer. The IGL from 45–60 days is composed of numerous, tightly packed, clearly visible granule cells and has at this point reached its greatest width and density.

TABLE III
Packing density and linear measurements

Age (days)	Packing density (mean number of cells/ /0.025 mm^2)				Linear measurements (mean width of layers at 0.01 mm)			
	EGL		IGL		EGL		IGL	
	Nodulus	Uvula	Nodulus	Uvula	Nodulus	Uvula	Nodulus	Uvula
60			18	19			0.25	0.22
55			15	14			0.24	0.22
50			15	14			0.22	0.22
45	10	10	14	14	0.01	0.01	0.22	0.17
40	10	11	13	14	0.01	0.02	0.20	0.16
35	14	14	13	13	0.02	0.02	0.20	0.16
30	13	12	13	13	0.03	0.03	0.20	0.16
26	20	21	13	12	0.07	0.07	0.19	0.16
20	23	24	13	12	0.11	0.09	0.18	0.14
15	28	29	13	12	0.11	0.10	0.17	0.14
10	20	22	12	10	0.11	0.11	0.17	0.13
5	21	25	11	12	0.11	0.12	0.13	0.13
0	19	19	10	11	0.13	0.11	0.13	0.11

Behavioral data

The results of the behavioral observations are summarized in Table IV. As indicated the young kitten possesses a full repertoire of adult balance and locomotor behaviors at the termination of the 1st month. It should be emphasized, however, that these behaviors are immature and are perfected during the 2nd postnatal month. The standard deviations reflect the variance that existed between litters with respect to the onset of these behaviors. It should be pointed out that this variability is largely a between litter occurrence, while within each litter all behaviors occurred in very close approximation to one another.

The newborn kitten shows at birth both the postural and crawl-approach reactions. It is through these behaviors that they were able to adjust their position to lie ventral side down and make gains toward the mother. The crawl-approach reaction may be considered the forerunner of walking and since both reactions appear full-blown minutes after birth, they may be considered genetically pre-programmed. Although the synergizing and eye-opening reactions commence during the first two weeks postnatally, the general mode of behavior characteristic of the neonatal kitten is lethargic. At the termination of the 2nd week young kittens are able to stand and the crawl-approach reaction gradually has given way to walking, although only a few steps can be taken before they lose their balance and fall. Vision is also apparent as seen by eye-focusing reactions. During the early part of the 3rd week young kittens were observed sitting although not completely upright. At the end of 3 weeks they began to climb up and out of their cages exploring the cattery and engaging in playful activity with one another. During

TABLE IV
Commencement of locomotor and balance patterns of behavior

Behavior pattern	Number of kittens	Age	
		Mean (days)	SD
Postural	15	birth	
Crawl-approach	15	birth	
Synergizing	12	7.4	0.49
Eye-opening	11	10.2	1.45
Anti-gravity	9	14.3	2.39
Walking	9	14.3	2.39
Eye-head turning	6	14.5	4.57
Sitting	9	16.6	1.71
Climbing	9	18.5	3.85
Running	7	21.7	5.80
Jumping	7	28.0	1.31

the 4th week they began to run, although they often lost their balance and fell, and could jump from a low lying surface but could not maintain their balance when landing on the ground.

The 2nd postnatal month was a period characterized by the perfection of the walking, running, climbing, and jumping reactions and a period of highly increased activity, exploration and development of self-sufficiency. A definite tremor in the hind legs persisted in all kittens observed until they were 35–40 days old. It was not until this tremor gradually ceased that the walking and running reactions were executed without loss of balance. During the latter part of the 2nd postnatal month, the young kittens had acquired the full repertoire of adult balance and locomotor patterns of behavior and had become self-sufficient.

DISCUSSION

Three major experimental approaches were undertaken to analyze the postnatal development of the feline cerebellum in connection with the developing balance and locomotor behavior patterns: (i) packing densities and linear measurements of the nodulus and uvula lobules of the vermis of the cerebellar cortex; (ii) qualitative analysis of changes in cell composition and changes in the morphological appearances of cell types; and (iii) behavioral observations of the development of locomotor activity. The results of these three experimental approaches confirmed the hypothesis that shortly after birth the external granular layer would commence rapid dissolution and that concomitant with this, there would be a gradual acquisition of smooth, skillful balance and locomotor activity.

The quantitative results obtained were in agreement with previous investigations involving mice (10), rats (1, 3), and dogs (12).

Qualitatively, the changes in cell composition and morphological appearances of cell types were in agreement with the histological and cytological observations discussed by Altman (3). We may consider 30th postnatal day as the major turning point in the development of the feline cerebellum. Whereas the 1st month is taken up with cell proliferation, the 2nd postnatal month is concerned with cell differentiation. It is at this point that the change in zonal composition of the EGL occurs. Prior to 30 days, round cells in the upper section of the layer predominate in numbers over the spindle-shaped cells found at its borders. Beyond 30 days, however, proportionally more spindle-shaped cells were found in the EGL. Further, the appearance of large numbers of vertically-oriented, spindle-shaped cells in the molecular layer is proportional to the dissolution of the EGL. That is, the greatest concentration occurs between 30–30 days postnatally at the same time that the EGL has changed in composition and become markedly reduced in size. Presumably, these spindle-shaped cells are the indifferent cells migrating towards the IGL.

Similarly, the packing density and the width of the IGL are continuing to rise; the packing density increases sharply after 40 days, some 10 days following the former transformation. Since these observations are in agreement with those of Altman (3) we may conclude that "the growth of the cerebellar cortex is directly or indirectly related to the migration of cells produced at a high rate in the subpial, external granular layer" (3, p. 285).

The results of the behavioral observations, in particular the commencement and development of the balance and locomotor activities, are in agreement with the findings of Tilney and Casamajor (14), Windle (15), and Tilney (13).

It is interesting to note the correlation between the persistence of tremor in the hind limbs of young kittens and the course of postnatal neurogenesis. This tremor is present at the onset of walking (14.3 days) and persists up to 35–40 days preventing the smooth execution of walking, running, and jumping by interfering with proper balance as the kittens move swiftly around corners, turn quickly, start or stop suddenly. That this tremor is indeed neurogenic is attested to by the fact that it is seen when the kitten is not supporting its own weight. At approximately one week before this ceases, the EGL has diminished and has changed in zonal composition; spindle-shaped cells are numerous in the molecular layer and the IGL is continuing to increase in both width and packing density. These results seem to indicate that the function of granule cells in the cerebellum is one of modulation and regulation in the execution of locomotor activity. Support for this conclusion is found in current research on the effects of the feline ataxia virus and the effects of ionizing radiation on the development of the cerebellar cortex (4, 9). Both the virus and X-irradiation specifically destroy newly forming postnatal cells leaving the cerebellum sparsely populated or devoid of microneurons, afflicting the animal with permanent locomotor ataxia (found in the former) or lasting subtle locomotor deficits (found in the latter) (4).

REFERENCES

1. ALTMAN, J. 1966. Autoradiographic and histological studies of postnatal neurogenesis. II. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in infant rats, with special reference to postnatal neurogenesis in some brain regions. J. Comp. Neurol. 128: 431–474.
2. ALTMAN, J. 1967. Postnatal growth and differentiation of the mammalian brain with implications for a morphological theory of memory. *In* G. C. Quarton, T. Melneckuk and F. O. Schmitt (ed.), The neurosciences: A study program. Rockefeller Univ. Press, New York, p. 723–743.

3. ALTMAN, J. 1969. Autoradiographic and histological studies of postnatal neurogenesis. III. Dating the time of production and onset of differentiation of cerebellar microneurons in rats. J. Comp. Neurol. 136: 269–294.
4. ALTMAN, J. 1970. Postnatal neurogenesis and the problem of neural plasticity. *In* W. A. Himwich (ed.), Developmental neurobiology. Charles C Thomas, Springfield, p. 197–273.
5. ALTMAN, J. and DAS, G. D. 1965. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J. Comp. Neurol. 124: 319–336.
6. ALTMAN, J. and DAS, G. D. 1966. Autoradiographic and histological studies of postnatal neurogenesis. I. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in neonate rats, with special reference to postnatal neurogenesis in some brain regions. J. Comp. Neurol. 126: 337–390.
7. DOW, R. S. and MORUZZI, G. 1958. The physiology and pathology of the cerebellum. Univ. Minnesota Press, Minneapolis. 675 p.
8. FOX, C. A. 1962. The structure of the cerebellar cortex. *In* E. C. Crosby, T. Humphrey and E. W. Laurer (ed.), Correlative anatomy of the nervous system. MacMillan, New York, p. 193–198.
9. KILHAM, L. and MARGOLIS, G. 1966. Viral etiology of spontaneous ataxis of cats. Amer. J. Pathol. 48: 991–1011.
10. MIALE, I. L. and SIDMAN, R. I. 1961. An autoradiographic analysis of histogenesis in the mouse cerebellum. Exp. Neurol. 4: 277–296.
11. OBERSTEINER, H. 1883. Der Feinere bau der Kleinhirnrinde bein Menschen und bei Tieren. Zentralbl. 15: 745–752.
12. PHEMISTER, R. D. and YOUNG, S. 1968. The postnatal development of the canine cerebellar cortex. J. Comp. Neurol. 134: 243–254.
13. TILNEY, F. 1933. Behavior in relation to the development of the brain. II. Correlation between the development of the brain and behavior in the albino rat from embryonic states to maturity. Bull. Neurol. Inst. N. Y. 3: 252–358.
14. TILNEY, F. and CASAMAJOR, L. 1924. Myelinization as applied to the study of behavior. Arch. Neurol. Psychiat. 12: 1–66.
15. WINDLE, W. F. 1930. Normal behavioral reactions of kittens correlated with the postnatal development of nerve fiber density in the spinal gray matter. J. Comp. Neurol. 50: 479–503.
16. ZEMAN, W. and INNES, J. R. M. 1963. Craigie's neuroanatomy of the rat. Acad. Press, New York. 230 p.

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