COO-2000-127

MASTER

FINAL REPORT:

**LOW-LEVEL X-IRRADIATION OF THE BRAIN DURING DEVELOPMENT
MORPHOLOGICAL, PHYSIOLOGICAL, AND BEHAVIORAL CONSEQUENCES**

Joseph Altman

**Department of Biological Sciences
Purdue University, W. Lafayette, Indiana**

September 1, 1976 - August 31, 1977

PREPARED FOR THE U. S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

TABLE OF CONTENTS

Page No.

1.	RESEARCH ACCOMPLISHMENTS	3
	(a) Glial recovery patterns in rat corpus callosum after x-irradiation during infancy	3
	(b) Cytogenesis and histogenesis of the deep nuclei and the cortex of the cerebellum	4
	(c) Cytogenesis and histogenesis of the inferior olive, pontine gray, and the precerebellar reticular nuclei	5
	(d) The postnatal development of the olfactory bulb	6
	(e) Time of origin and distribution of a new cell type in the rat cerebellar cortex	7
2.	BIBLIOGRAPHY, 1976-77	8 & 9
3.	TRAINING FUNCTIONS, 1976-77	10

1. RESEARCH ACCOMPLISHMENTS

In the terminal year of this contract morphological research has been continued in the following areas: (1) glial recovery patterns in the rat corpus callosum after x-irradiation during infancy; (2) the prenatal development of the deep nuclei and cortex of the cerebellum; (3) the prenatal development of the inferior olive, pontine gray and the precerebellar reticular nuclei; and (4) the postnatal development of the olfactory bulb. In these studies autoradiography and x-irradiation were among the experimental techniques utilized. The behavioral studies, all of which are still in progress, are concerned with the effects of different schedules of postnatal x-irradiation of the cerebellum, and the effects of x-irradiation of the olfactory bulb. This summary is restricted to completed studies, either published, in print, or submitted for publication (see Bibliography, 1976-77). An incidental study involved the identification of a new cell type in the rat cerebellar cortex.

(a) GLIAL RECOVERY PATTERNS IN RAT CORPUS CALLOSUM AFTER X IRRADIATION DURING INFANCY

Beginning on Day 2 after birth the head region containing the corpus callosum in three groups of male rat pups was exposed two (2X), four (4X) or eight (E8X) times to low-dosage X radiation (150 to 200 R) on successive or alternate days. A fourth group of male rat pups was started on a series of eight exposures on Day 8 (L8X). The effects of irradiation-induced interference with glial cell accumulation were assessed at 30, 60, 120, and 200 days of age by determining the number of cells per unit area, total area, and total number of cells in anatomically matched sagittal sections. The 2X and 4X groups had control values in all parameters at 30 and 60 days, which tended to decrease by 120 and 200 days. In contrast, a marked reduction in all parameters was seen in both E8X and L8X groups at 30 days. By either 60 (E8X) or 120 (L8X) days, there was recovery to control values in cells per unit area. Both total area and total number of cells showed only partial recovery in the 8X groups. The initial effect on only the

Contract No. EY-76-S-02-2000. *000

8X groups is due to the exposures extending into the third week when gliogenesis is at its peak. The delayed effect in all groups may be a suppression of the normal increase in glial cells of the corpus callosum which occurs during the adult period.

(b) CYTOGENESIS AND HISTOGENESIS OF THE DEEP NUCLEI AND THE CORTEX OF THE CEREBELLUM

Prenatal cerebellar development was investigated with three approaches. In normal embryos sectioned in three planes morphological and cytological changes were determined at daily intervals beginning on embryonic day 13 (E13). A similar series of x-irradiated embryos was used to study changes in neuro-epithelial organization and in the location of primitive (radiosensitive) or differentiated cells. Finally, to quantify the time of origin of different classes of cerebellar neurons with the progressively delayed labelling procedure, we used autoradiograms from adult rats whose mothers were injected with two successive daily doses of ^3H -thymidine on overlapping days from day E13 on.

The cerebellar anlage was delineated in the dorsal metencephalon by the collapse of its ventricular lining after x-irradiation. This "collapsing neuroepithelium" was located laterally on day E13, then it spread medially and reached the midline on day E16. Deep nuclear neurons began to differentiate on day E13, with two-thirds forming on day E14; Purkinje cell formation peaked on day E15, with a few cells still forming on day E16. It was postulated that the deep nuclear neurons settled first in the superficial "nuclear zone", and that the Purkinje cells gathered temporarily in the underlying "transitory zone", adjacent to the collapsing neuroepithelium.

In the next period of cerebellar development four major events were recognized. (1) Beginning on day E17 the cells of the nuclear and transitory zones became intermingled. It was postulated that the Purkinje cells were migrating radially through the ranks of the stationary deep nuclear neurons and assembled under the spreading canopy of a fibrous plexus and the external germinal layer. (2) It was also on day E17 that the external germinal layer began to form as one of the prongs of the "germinal trigone" in the posteroventral aspect of the cerebellum.

Contract No. EY-76-S-02-2000. *000

On the succeeding days the external germinal layer spread over the surface of the cerebellum; in the vermis in a rostral direction. (3) Two cell types destined to settle in the future granular layer, the pale cells and the Golgi cells, began to form at a relatively slow rate on day E19. Chronological considerations suggested that they were generated in the regressing, noncollapsing neuroepithelium of the cerebellar ventricle. (4) From the beginning (day E17) of its genesis posteroventrally, the primitive cerebellar cortex bridged the midline. As the fused cortex spread, the vertical ventricular cleft separating the underlying portions of the cerebellum became shallower and then disappeared; the process was completed in the anterior cerebellum by day E22. By the time of birth the maturation of the neurons of the deep nuclei appeared advanced but the maturation of the prenatally produced neurons of the cortex does not start until after birth when a new class of neurons is generated in the external germinal layer.

(c) CYTOGENESIS AND HISTOGENESIS OF THE INFERIOR OLIVE, PONTINE GRAY, AND THE PRECEREBELLAR RETICULAR NUCLEI

Aspects of the prenatal development of the inferior olive, lateral reticular nucleus, nucleus reticularis tegmenti pontis, and of the pontine gray were investigated in the rat. The material used was the same as in the preceding study of cerebellar development, including histological preparations from normal and x-irradiated embryos at daily intervals (days E13 to E22), and autoradiograms from adult rats injected with two successive doses of ^3H -thymidine on overlapping days from days E13+E14 on.

Neurons of the inferior olive form on days E13 and 14: those settling rostrally in the principal nucleus somewhat ahead of those settling in the medial accessory nucleus caudally. The olivary neurons are generated in the neuroepithelium of the lateral recess of the fourth ventricle, migrate ventrally by way of the olivary migratory stream, split into two branches and provide cells to the olivary complex from its lateral and medial aspect. The migrating cells are differentiated, radioresistant elements. It was postulated that since the olivary migratory path and the course of olivary fibers (the inferior cerebellar peduncle) are on the same trajectory, climbing fibers could reach the surface of the cerebellum before the

Contract No. EY-76-S-02-2000. *000

arrival of the radially migrating Purkinje cells.

Neurons of the lateral reticular nucleus form predominantly on days E13 and 14, of the nucleus reticularis tegmenti pontis on days E15-17, and of the pontine gray on days E17-19. The pontine neurons originate several days after but at the same site as the olivary neurons. The cells migrating in the pontine migratory stream are undifferentiated elements and follow a course different to that of their prospective axons in the middle cerebellar peduncle. The earliest forming pontine neurons settle near the pyramidal tract, the later arriving cells form cap-like shells around this core. Axonogenesis of pontine cells begins after the settling of the perikarya on day E19. The middle cerebellar peduncle appeared to approach the cerebellum on day E22, suggesting that pontine mossy fibers do not establish contact with cerebellar elements before the perinatal period, by which time the external germinal layer and the Purkinje cells have formed a cortical mantle over the entire cerebellum.

In an attempt to correlate the chronology of cytogenesis in some of the precerebellar nuclei with cell production and maturation in the cerebellum, it was hypothesized that mossy fibers of reticular origin exert influence on Purkinje cell dendrites in the lower parts of the molecular layer, whereas mossy fibers of pontine origin influence the upper parts of Purkinje cell dendrites. Reference is made to behavioral results indicating the mediation of dissociable functions by elements of the lower and upper molecular layer.

(d) THE POSTNATAL DEVELOPMENT OF THE OLFACTORY BULB

The postnatal development of the main olfactory bulb was examined quantitatively from birth to one year. The volume of the main olfactory bulb increased over seven-fold by day 30 and remained unchanged thereafter. This pattern was chronologically paralleled by the volume of the granular layer, which increased 18-fold during this period, and by the area of the olfactory glomerulus, which increased seven-fold. Mitral cell perikaryal area doubled between the neonatal and juvenile periods but their total number declined between days 6 and 20.

In the internal granular layer (IGL) of the main olfactory bulb 89 per cent of the granule cells were acquired postnatally. Much of the cellular gain occurred during the first three weeks, with the period of maximum acquisition between days

Contract No. EY-76-S-02-2000. *000

8 and 14. The number of subependymal cells, the precursors of granule cells, reached a peak at 12 days and gradually declined. But some primitive cells could still be found at one year of age and there was an increase in the total number of granule cells beyond day 30. The mean number of IGL cells in a single main olfactory bulb of adult rats was about 5 million; the number of mitral cells about 40 thousand.

In the animals injected with thymidine-³H on day 20 and killed 2 hours after injection a small but significant proportion of cells was labelled in the subependymal layer but few in the IGL. In the animals killed 20 and 40 days after injection there was a 10-11-fold rise in the proportion of labelled IGL cells. In the sections examined there was also an increase in the number of granule cells between the ages of day 40 and 360, but with no significant increases after day 90. The relatively few cells acquired after day 90 were equally derived from labelled and unlabelled precursors.

(e) TIME OF ORIGIN AND DISTRIBUTION OF A NEW CELL TYPE IN THE RAT CEREBELLAR CORTEX

A new cell type was identified in the granular layer of the rat cerebellum. It has a lightly staining nucleus with a nucleolus, it is spherical in shape and is larger than granule cells and smaller than Golgi cells. These pale cells are preferentially concentrated in the nodulus, the ventral uvula, the lingula, the flocculus, and parts of the paraflocculus. According to autoradiographic (³H-thymidine) evidence, over 60 per cent of these pale cells are formed on embryonic days 19 and 20, and their production comes to an end soon after birth, prior to the differentiation of granule cells. The possible relation of pale cells to vestibular afferents of the cerebellar cortex was discussed (11).

Contract No. EY-76-S-02-2000. *000

2. BIBLIOGRAPHY, 1976-1977

1976

1. Altman, J., 1976 Experimental reorganization of the cerebellar cortex: V. Effects of early X-irradiation schedules that allow or prevent the acquisition of basket cells. J. Comp. Neurol., 165: 31-48.
2. Altman, J., 1976 Experimental reorganization of the cerebellar cortex: VI. Effects of X-irradiation schedules that allow or prevent cell acquisition after basket cells are formed. J. Comp. Neurol., 165: 49-64.
3. Altman, J., 1976 Experimental reorganization of the cerebellar cortex: VII. Effects of late X-irradiation schedules that interfere with cell acquisition after stellate cells are formed. J. Comp. Neurol., 165: 65-76.
4. Altman, J., 1976 Development of the Purkinje cell dendritic system: The role of basket, stellate and granule cell axons. Exp. Brain Res. Suppl. 1: 26-30.
5. Altman, J. and F. G. Bulut, 1976 Organic maturation and the development of learning capacity; Exploratory studies. In, Neural Mechanisms of Learning and Memory, M. R. Rosenzweig (Ed.), MIT Press, pp. 236-240.
6. McBride, W. J., N. S. Nadi, J. Altman and M. H. Aprison, 1976 Effects of selective doses of X-irradiation on the levels of several amino acids in the cerebellum of the rat. Neurochem. Res., 1: 141-152.

1977

7. Altman, J. and A. T. Winfree, 1977 Postnatal development of the cerebellar cortex in the rat: V. Spatial organization of Purkinje cell perikarya. J. Comp. Neurol., 171: 1-16.
8. Bauer-Moffett, C. and J. Altman, 1977 The effect of ethanol chronically administered to preweanling rats on cerebellar growth. Brain Res., 119: 249-268.

Contract No. EY-76-S-02-2000. *000

9. Bayer, S. A., 1977 Glial recovery patterns in rat corpus callosum after X-irradiation during infancy. Exp. Neurol., 54: 209-216.
10. Bayer, S. A. and P. J. Peters, 1977 A method for X-irradiating selected brain regions in infant rats. Brain Res. Bull., 2:153-156.
11. Altman, J. and S. A. Bayer (in press) Time of origin and distribution of a new cell type in the rat cerebellar cortex. Exp. Brain Res., 29: 1-10.
12. Altman, J. and S. A. Bayer (submitted for publication) Prenatal development of the cerebellar system in the rat: I. Cytogenesis and histogenesis of the deep nuclei and the cortex of the cerebellum. J. Comp. Neurol.
13. Altman, J. and S. A. Bayer (submitted for publication) Prenatal development of the cerebellar system in the rat: II. Cytogenesis and histogenesis of the inferior olive, pontine gray and the precerebellar reticular nuclei. J. Comp. Neurol.
14. Rosselli-Austin, L. and J. Altman (submitted for publication) The postnatal development of the main olfactory bulb of the rat. Exp. Brain Res.

Contract No. EY-76-S-02-2000. *000

3. TRAINING FUNCTIONS, 1976-1977

GRADUATE STUDENTS TRAINED:

C. Bauer-Moffett (Ph.D. 1976)
D. Barnes (Ph.D. 1977)
F. Bulut (Ph.D. 1977)
V. Wootten (Ph.D. 1977)
K. Browne
R. Gazzara
J. Gruner
K. Jensen

POSTDOCTORAL FELLOWS TRAINED:

Dr. L. Rosselli-Austin 1975-76
(Now at Chicago State University)

Dr. J. Yanai 1975-76
(Now at Illinois Medical Center)

Dr. C. Walters 1976-78
Dr. N. Laughlin 1976-77
Dr. R. Strubel 1977-78