EFFECTS OF LATE PRENATAL IRRADIATION ON THE DEVELOPMENT OF THE CEREBELLAR CORTEX IN THE RAT

by

TERESA ANNA MARIA RALCEWICZ

A Thesis

Submitted to the Faculty of Graduate Studies

In partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

UNIVERSITY OF MANITOBA
FACULTY OF MEDICINE
DEPARTMENT OF ANATOMY

Winnipeg, Manitoba
CANADA

May 1994

ISBN 0-315-98970-X

Name TERESA ANNA MARIA RALCEWICZ

Dissertation Abstracts International is arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation. Enter the corresponding four-digit code in the spaces provided.

NEUROSCIENCE

SUBJECT TERM

0317 U·M·I

SUBJECT CODE

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS
Architecture 0729
Art History 0377
Cinema 0900
Dance 0378
Fine Arts 0357
Information Science 0723
Journalism 0391
Library Science 0399
Mass Communications 0708
Music 0413
Speech Communication 0459
Theater 0465

EDUCATION
General 0515
Administration 0514
Adult and Continuing 0516
Agricultural 0517
Art 0273
Bilingual and Multicultural 0282
Business 0688
Community College 0275
Curriculum and Instruction 0727
Early Childhood 0518
Elementary 0524
Finance 0277
Guidance and Counseling 0519
Health 0680
Higher 0745
History of 0520
Home Economics 0278
Industrial 0521
Language and Literature 0279
Mathematics 0280
Music 0522
Philosophy of 0998
Physical 0523
Psychology 0525
Reading 0535
Religious 0527
Sciences 0714
Secondary 0533
Social Sciences 0534
Sociology of 0340
Special 0529
Teacher Training 0530
Technology 0710
Tests and Measurements 0288
Vocational 0747

LANGUAGE, LITERATURE AND LINGUISTICS
Language
- General 0679
- Ancient 0289
- Linguistics 0290
- Modern 0291

Literature
- General 0401
- Classical 0294
- Comparative 0295
- Medieval 0297
- Modern 0298
- African 0316
- American 0591
- Asian 0305
- Canadian (English) 0352
- Canadian (French) 0355
- English 0593
- Germanic 0311
- Latin American 0312
- Middle Eastern 0315
- Romance 0313
- Slavic and East European 0314

PHILOSOPHY, RELIGION AND THEOLOGY
Philosophy 0422
Religion
- General 0318
- Biblical Studies 0321
- Clergy 0319
- History of 0320
- Philosophy of 0322

Theology 0469

SOCIAL SCIENCES
American Studies 0323
Anthropology
- Archaeology 0324
- Cultural 0326
- Physical 0327

Business Administration
- General 0310
- Accounting 0272
- Banking 0770
- Management 0454
- Marketing 0338

Canadian Studies 0385
Economics
- General 0501
- Agricultural 0503
- Commerce-Business 0505
- Finance 0508
- History 0509
- Labor 0510
- Theory 0511

Folklore 0358
Geography 0366
Gerontology 0351
History
- General 0578
- Ancient 0579
- Medieval 0581
- Modern 0582
- Black 0328
- African 0331
- Asia, Australia and Oceania 0332
- Canadian 0334
- European 0335
- Latin American 0336
- Middle Eastern 0333
- United States 0337

History of Science 0585
Law 0398
Political Science
- General 0615
- International Law and Relations 0616
- Public Administration 0617

Recreation 0814
Social Work 0452
Sociology
- General 0626
- Criminology and Penology 0627
- Demography 0938
- Ethnic and Racial Studies 0631
- Individual and Family Studies 0628
- Industrial and Labor Relations 0629
- Public and Social Welfare 0630
- Social Structure and Development 0700
- Theory and Methods 0344

Transportation 0709
Urban and Regional Planning 0999
Women's Studies 0453

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES
Agriculture
- General 0473
- Agronomy 0285
- Animal Culture and Nutrition 0475
- Animal Pathology 0476
- Food Science and Technology 0359
- Forestry and Wildlife 0478
- Plant Culture 0479
- Plant Pathology 0480
- Plant Physiology 0817
- Range Management 0777
- Wood Technology 0746

Biology
- General 0306
- Anatomy 0287
- Biostatistics 0308
- Botany 0309
- Cell 0379
- Ecology 0329
- Entomology 0353
- Genetics 0369
- Limnology 0793
- Microbiology 0410
- Molecular 0307
- Neuroscience 0317
- Oceanography 0416
- Physiology 0433
- Radiation 0821
- Veterinary Science 0778
- Zoology 0472

Biophysics
- General 0786
- Medical 0760

EARTH SCIENCES
Biogeochemistry 0425
Geochemistry 0996
Geodesy 0370
Geology 0372
Geophysics 0373
Hydrology 0388
Mineralogy 0411
Paleobotany 0345
Paleoecology 0426
Paleontology 0418
Paleozoology 0985
Palynology 0427
Physical Geography 0368
Physical Oceanography 0415

HEALTH AND ENVIRONMENTAL SCIENCES
Environmental Sciences 0768
Health Sciences
- General 0566
- Audiology 0300
- Chemotherapy 0992
- Dentistry 0567
- Education 0350
- Hospital Management 0769
- Human Development 0758
- Immunology 0982
- Medicine and Surgery 0564
- Mental Health 0347
- Nursing 0569
- Nutrition 0570
- Obstetrics and Gynecology 0380
- Occupational Health and Therapy 0354
- Ophthalmology 0381
- Pathology 0571
- Pharmacology 0419
- Pharmacy 0572
- Physical Therapy 0382
- Public Health 0573
- Radiology 0574
- Recreation 0575
- Speech Pathology 0460
- Toxicology 0383

Home Economics 0386

PHYSICAL SCIENCES
Pure Sciences
Chemistry
- General 0485
- Agricultural 0749
- Analytical 0486
- Biochemistry 0487
- Inorganic 0488
- Nuclear 0738
- Organic 0490
- Pharmaceutical 0491
- Physical 0494
- Polymer 0495
- Radiation 0754

Mathematics 0405
Physics
- General 0605
- Acoustics 0986
- Astronomy and Astrophysics 0606
- Atmospheric Science 0608
- Atomic 0748
- Electronics and Electricity 0607
- Elementary Particles and High Energy 0798
- Fluid and Plasma 0759
- Molecular 0609
- Nuclear 0610
- Optics 0752
- Radiation 0756
- Solid State 0611

Statistics 0463

Applied Sciences
Applied Mechanics 0346
Computer Science 0984
Engineering
- General 0537
- Aerospace 0538
- Agricultural 0539
- Automotive 0540
- Biomedical 0541
- Chemical 0542
- Civil 0543
- Electronics and Electrical 0544
- Heat and Thermodynamics 0348
- Hydraulic 0545
- Industrial 0546
- Marine 0547
- Materials Science 0794
- Mechanical 0548
- Metallurgy 0743
- Mining 0551
- Nuclear 0552
- Packaging 0549
- Petroleum 0765
- Sanitary and Municipal 0554
- System Science 0790

Geotechnology 0428
Operations Research 0796
Plastics Technology 0795
Textile Technology 0994

PSYCHOLOGY
General 0621
Behavioral 0384
Clinical 0622
Developmental 0620
Experimental 0623
Industrial 0624
Personality 0625
Physiological 0989
Psychobiology 0349
Psychometrics 0632
Social 0451

EFFECTS OF LATE PRENATAL IRRADIATION ON THE DEVELOPMENT OF THE CEREBELLAR CORTEX IN THE RAT

BY

TERESA ANNA MARIA RALCEWICZ

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

TABLE OF CONTENTS

DEDICATION

I thank God for two important people in my life to whom I dedicate this thesis. Throughout the duration of my doctoral thesis they have believed in me, provided support and encouragement, and have always been there for me.

To my mother Antonina,

for "All I am, or hope to be, I owe to my angel Mother"

A. Lincoln.

and

To my brother Henryk,

who has reaffirmed in me that
"Dreams are the touchstones of our character"

H.D. Thoreau

and

"A journey of a thousand miles
must begin with a single step"

Lao-Tzu

ACKNOWLEDGMENTS

Firstly, I would like to thank my supervisor Dr. T.V.N. Persaud for his support, advice and criticism throughout this study. I would also like to thank Dr. J.E. Bruni and the members of my advisory committee for their suggestions and comments: Dr. C. Pinsky, Dr. D. Nance, Dr. K. Jones and Dr. G. Froese.

Throughout the course of my doctoral studies, there are other individuals whom I would like to thank: Fran Thompson for her assistance with word processing and for helping me with the typing of this thesis; Roy Simpson for his suggestions with respect to the photographs and illustrations; Paul Perumal and Bill Pylypas for their assistance with the histological techniques; Gloria Gramek, Department Head of Medical Laboratory at Red River Community College for her encouragement and support in completing this thesis; Don Carlson from Medical Laboratory at Red River Community College for his time and assistance with various computer programs.

Finally, I would like to thank the members of the Anatomy Department for their friendship.

I would like to thank the Department of Anatomy for two Anatomical Research Scholarships that were awarded during the tenure of my studies.

This research was supported by funds from the Atomic Energy Control Board and from Health and Welfare, Canada.

ABSTRACT

The effects of maternal exposure to a single dose of whole body irradiation (0.5 Gy) on gestational days (GD) 17, 18, 19, or 20 on the development of the cerebellum was examined in the offspring of Sprague Dawley rats at 21 and 28 days postnatally. No gross cerebellar anomalies were observed in the irradiated animals. However, rats irradiated on each of GD-17, 18, 19 and 20 showed a significant incidence ($p < 0.05$) of circumscribed cerebellar lesions (CL) distributed in the inner granular layer of the anterior and posterior lobes. These lesions were characterized by a loss of granule cells and atrophied and/or reduced number of Purkinje cells. In 21 day old rats, irradiation on GD-17 resulted in more CL anteriorly (75%) and in the vermis whereas on GD-20, the CL predominated posteriorly (100%) and in the lateral hemispheres. In 28 day old rats, following irradiation on each of GD-17 and GD-20, there was an equal distribution of CL in both the anterior and posterior lobes. However, with irradiation on both GD-17 and GD-20, CL occurred more frequently in the anterior lobe of the lateral hemispheres, whereas in the posterior lobe they predominated in the vermis.

The laterolateral numbers of both granule and Purkinje cells in the pyramis were significantly reduced ($p < 0.001$) from controls in rats irradiated on each of GD- 17, 18, 19 and 20. There was a greater deficit in granule cell

number with irradiation on GD-20 than on GD-17 ($p < 0.05$). Purkinje cells were reduced in number with irradiation on GD-17 and GD-20; however, the decrease did not correspond to the degree of reduction in the number of granule cells. There was a greater reduction of both granule and Purkinje cells in the vermis with irradiation on GD-17, whereas on GD-20, both granule ($p < 0.05$) and Purkinje cells ($p < 0.001$) were more reduced in the lateral hemispheres. The GC/PC ratio was smaller in rats irradiated on GD-20 than on GD-17. The GC/PC ratio between the irradiated animals and the controls were relatively similar.

The results from this study suggest that a direct relationship exists between the proliferation, migration, development, and maturation of granule cells and their induction by Purkinje cells. The findings also support the view that both cell death and the regulation of granule cells by Purkinje cells maximize the effective development and organization of the cerebellum.

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

GD	Gestational Day
CL	Cerebellar Lesion
PC	Purkinje Cells
GC	Granule Cells
PN	Postnatal
ED	Embryonic Day
PCL	Purkinje Cell Layer
IGL	Inner Granular Layer
ML	Molecular Layer
MC	Medullary Center

1.0 HISTORICAL PERSPECTIVE AND CURRENT INVESTIGATION

The reporting by Roentgen in 1895 to the Wurzburg Physico-Medical Society on a new form of radiation and the discovery of radioactivity by Becquerel in 1896 resulted in the birth of radiobiology. Radiobiology is a branch of science concerned with the action of light, ultraviolet and ionizing radiation on living organisms (Dorlands 1982). It is also characterized by the 4 R's: 1) repair of sublethal damage, 2) repopulation and regrowth of cells, 3) redistribution of cells within the cell cycle, and 4) reoxygenation (Hall 1978m).

After the discovery of X-rays, many of the investigators in radiotherapy who exposed their hands to irradiation for several hours developed radiation induced cancers and died. The one survivor, Hall-Edwards, was the first to record carcinomas as a direct result from exposure to X-rays (Furth & Lorenz 1954). In earlier years, radiation was used to perform therapeutic abortion. The center of the uterus was irradiated with 360 rad no later than the fourteenth week of gestation (Yamazaki 1966, Mayer et. al. 1936). In 90% of the cases, the conceptus aborted spontaneously.

During the late 1910's and throughout the 1920's and 1930's, several investigators reported a variety of fetal abnormalities in rats and rabbits with exposure to prenatal irradiation (Bagg 1922, Murphy & de Renyi 1930, Hanson 1923). The earliest animal work lacked the precise timing of the

stage of development when irradiation was done. In 1935, Job, Leibold, and Fitzmaurice were the first to determine critical periods in development and were able to correlate the defects with the time of irradiation. The discovery of critical periods, prompted other investigators including Kosaka (1927), Warkany and Schraffenberger (1947), Wilson and his co-workers (1951, 1953, 1954) to study the relation between the timing of irradiation and the resultant abnormalities.

In an extensive series of experiments, Russell (1950) defined the critical periods for the induction of malformations by radiation in mice. She correlated the induction of external and gross visceral changes with the precise timing of irradiation during a particular stage of development. Through an extensive series of experiments, Altman, Hicks and each of their co-workers, have used radiation to study the progressive stages of cerebellar neurogenesis in rats from birth to adolescence. They have used radiation as an experimental tool to study radiation induced morphogenetic changes and re-organization of cerebellar constituents following irradiation during specific stages in development (Altman, Altman et. al. 1965-69, 1971-73, 1976-78; Hicks, Hicks et. al. 1950, 1952-58, 1980). Using radiation as an experimental tool, has allowed them to draw inferences about normal patterns of neurological development of the nervous system in mammals based on their studies of the malformation processes.