Histol Histopathol (1995) 10: 371-383

Histology and Histopathology

Effects of prenatal exposure to low dose ionizing radiation on the development of the cerebellar cortex in the rat

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Summary. 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 cerebellar cortex 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, compared to control animals, rat irradiated on each of GD-17, 18, 19 and 20 showed a significantly higher 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, these CL occurred more frequently in the lateral hemispheres of the anterior lobe, whereas in the posterior lobe they predominated in the vermis.

These results 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.

Key words: Irradiation, Brain development, Cerebellar lesions, Internal granular layer, Rat

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Introduction

Radiation is an established teratogen (Brent, 1979, 1980; Hicks and D'Amato, 1980; Schull et al., 1990), and both animal and human data have shown that radiation has a direct effect on the brain. Several investigators (Altman, 1972a,b,c; Altman and Anderson, 1972; Altman and Bayer, 1978a,b; Hicks and D'Amato, 1980) have used radiation as an experimental tool to investigate radiation induced morphogenetic changes and reorganization of cerebellar constituents following irradiation during specific stages in development. Some insights about normal patterns of neurological development in mammals have been gained from the results of these studies.

It is well documented that in animals, prenatal exposure to ionizing radiation (>1 Gy) results in intrauterine growth retardation, genetic defects, anomalies of the developing nervous system, deficits in motor coordination, hyperactivity and a hoping gait, increased emotional behavior, deficits in maze learning, death of neuronal or glial precursors, altered migratory pathways, degeneration of post-mitotic neurons, altered cell surface contact and synaptogenesis, as well as disoriented dendritic arborizations to name a few (Fowler et al., 1962; Werboff et al., 1962; Norton et al., 1976; D'Amato and Hicks, 1980; Hicks and D'Amato, 1980; Jensh and Brent, 1986, 1987, 1988; Jensh et al., 1987; Kimler and Norton, 1988; Norton, 1989).

The CNS has a long lasting sensitive period extending from the beginning of organogenesis to the neonatal period (Kameyama and Hoshino, 1986). The cerebellar anlage appears on embryonic day 13 (ED-13) when the neuroepithelium of the paired dorsal metencephalic plates (DMP) begins to collapse (Altman and Bayer, 1978b). In rats, histogenesis of the macroneurons of the cerebellum extends from embryonic day 13 to 15 when the Purkinje and the Golgi cells are produced followed by their migration. The

microneurons are produced from the external germinal layer; granule, basket and stellate cells arise postnatally from day 4 through day 21. Since maturation of the cerebellum occurs largely postnatally, the sensitive phase of its histogenetic components extends from embryonic day 14 through postnatal day 21 (Altman and Das, 1965; Altman, 1972a,b,c; Altman and Bayer, 1978b). Irradiation during this period results in no prenatal death, but in various cerebellar defects (Hicks, 1954).

At birth, the cerebellum is largely immature and postnatal development is characterized by intense cell proliferation, migration and differentiation. Because the development of locomotory skills is correlated with postnatal cerebellar neurogenesis, any prenatal insult can be manifested visibly postnatally by deficits in motor function. Any alterations from norm, such as growth alterations, cerebellar lesions or hypoplasia as a result from exposure in utero to irradiation will be characterized by ataxia with a loss of motor coordination as a result of the paucity of granule cells or interference of connections between Purkinje and granule cells (Altman et al., 1968).

There is little information concerning the effects of maternal exposure to low levels of ionizing radiation (<1 Gy) during late gestation on the postnatal development of the cerebellum. This study examines the effects of maternal exposure to a single dose of whole body irradiation (0.5 Gy) on gestational days (GD) 17, 18, 19, and 20 on the development of the cerebella in the offspring of Sprague-Dawley rats at 21 and 28 days of life.

Materials and methods

The offspring of 36 Sprague-Dawley rats, totalling 288 animals, were used in this study. The rats were cared for in accordance with the guidelines set by the Canadian Council of Animal Care. The rats were housed in wire mesh cages and maintained under controlled room temperatures (20±2 °C) and illumination (12 hour light/dark cycle, 2000-0800 dark). Water and laboratory rat chow were given *ad libitum*. Timed pregnancies were obtained by housing two females (between 200-225 g) with a male rat of the same strain overnight. The following day, the females were checked for vaginal plugs and vaginal smears were obtained. If the females showed sperm-positive smears, they were designated as gestational day 1 (GD-1). Pregnant females were housed individually in plastic cages with bedding. Groups of pregnant rats, (N= 3-6/group), were randomly allocated to either a control or treatment group. Within each group, several pregnant rats were killed either 21 or 28 days postnatally. Within 36 hours after parturition, all litters in each group (control or treatment) were reduced to 8 neonates.

Between 1600-1700 hours, pregnant rats were placed in a specially constructed 20x10x7 cm clear lucite plexiglass cage, divided into two equal compartments. The rats were exposed to 50 cGy or 50 Rad (1 Gray = 100 Rad) of 60 Co gamma radiation using a Theratron F Cobalt Radiotherapy Unit. To ensure uniform dose distribution throughout the irradiated volume, the animals were exposed to parallel opposed radiation fields (antero-posterior and postero-anterior), positioned 75 cm above or below the surface of the plexiglass cage on the treatment table. The time of exposure for both fields was calculated at 16.5 seconds. Total time of confinement was 20-30 minutes. Control animals were treated in the same manner except that they were not irradiated. All irradiation experiments were completed within a 12 month period.

At 21 or 28 days PN, the rats were anesthetized with an intraperitoneal injection of Nembutal (50 mg/Kg). While under anesthesia, the chest cavity was opened and the animals were perfused transcardially with 2.5% glutaraldehyde-2% paraformaldehyde in 0.12 M phosphate buffer and 0.02 mM calcium chloride. The 37 °C fixative was delivered from a column height of 110-115 cm for a period of 15-20 minutes. Following perfusion, the brains were excised, examined for gross anomalies and stored overnight in a fresh solution with the same fixative at 4 °C.

For light microscopic examination (LM), a representative number of brains (N= 10 or 12/group) were randomly selected for processing. The tissues were rinsed with a rinse solution (0.12M phosphate buffer with 0.02 mM calcium chloride and 8% dextrose). The tissues not immediately used were placed in this dextrose rinse solution which was changed every two to three weeks to prevent growth of mold.

The brains were processed with chloroform and the cerebella were serially sectioned at 5 µm from rostral to caudal in the coronal pale with 100 µm intervals between sections. Every third to fifth section was stained with thionin.

Differences between the cerebella of control and irradiated animals were analyzed statistically using Fisher's exact and chi-square tests and ANOVA.

Results

Rats irradiated with 0.5 Gy on gestational days (GD) 17, 18, 19, or 20 and examined at either 21 or 28 days postnatally exhibited hyperactivity, spasticity and a hopping gait. This motor deficit was noted in all irradiated groups; however, the walking patterns were only observed and not recorded.

The cerebella of rats irradiated on GD-17, 18, 19, or 20 and examined at either 21 or 28 days postnatal, exhibited no gross anomalies and were normal in size, folial, sulci and fissure formation. Within the individual lobules there was variation in the size and contour of the molecular layer but this was observed in both control and all irradiated rats. The trilaminar cortical architecture was maintained and well defined in the cerebella of both control (Fig. 1) and all groups of irradiated rats (Fig. 2). The most conspicuous change in the cortices of rats irradiated between GD-17, 18, 19 and

20 was the presence of several circumscribed cerebellar lesions (CL) throughout the inner granular layer (Fig. 2). These lesions were characterized by reduced granule cell numbers and condensation and/or atrophy of some of the remaining granule cells (Figs. 4, 6).

The most striking changes in the Purkinje cell layer

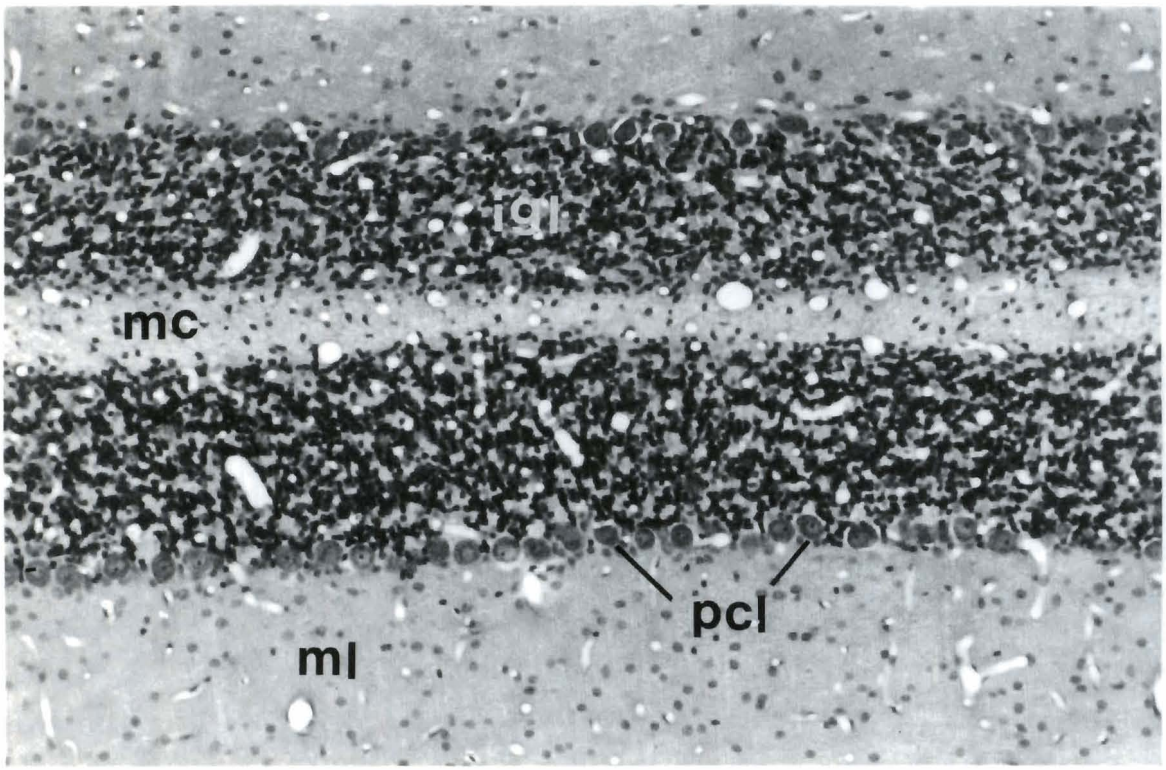

Fig. 1. Coronal section of the cerebellar folia from a 28-day old non-irradiated control rat illustrating the normal trilaminar cortical architecture. ML: molecular layer; PCL: Purkinje cell layer; IGL: inner granular layer; MC: medullary center. Thionin stained. x 40

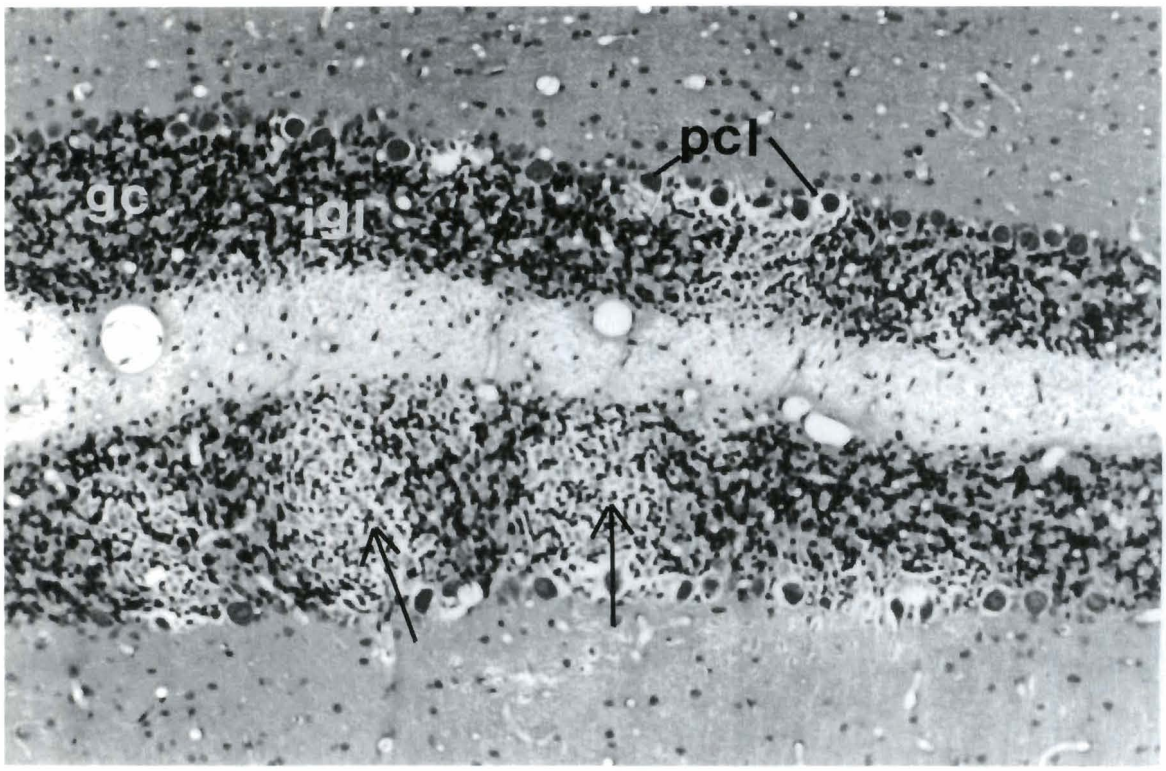

Fig. 2. Cerebellum of a 28 day old rat irradiated with 0.5 Gy on GD-17 illustrating the circumscribed lesions (solid arrows) distributed amongst the population of normal appearing granule cells within the inner granular layer. Coronal section. IGL: inner granular layer; PCL: Purkinje cell layer. Thionin stained. x 40

following irradiation was the presence of gaps, variable in length, and atrophied cells. These gaps and/or atrophy of Purkinje cells were present immediately above the lesions in the inner granular layer (Figs. 3-6). Occasionally, Purkinje cells were found in the inner granular layer scattered amongst the granule cells. The

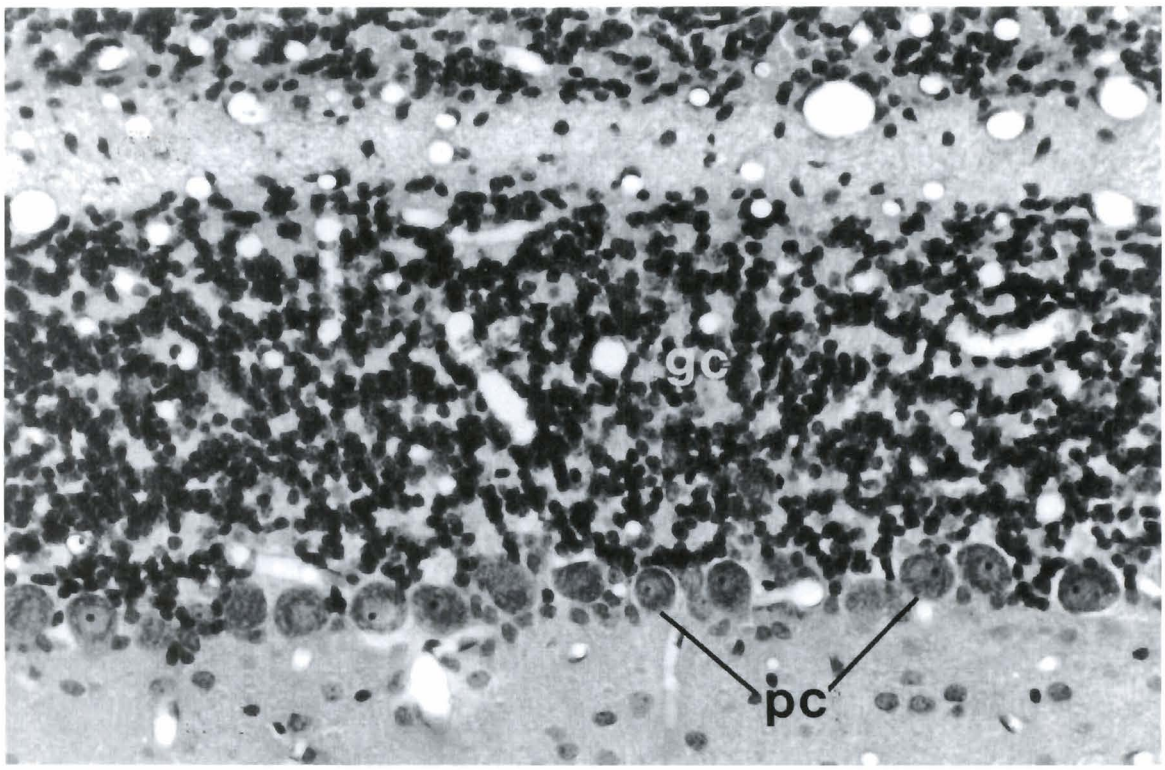

Fig. 3. Coronal section of the inner granular layer from the cerebellum of a 28 day old non-irradiated rat illustrating the normal population of granule cells clustered beneath the Purkinje cells. GC: granule cells; PC: Purkinje cells. A higher magnification of Figure 1. Thionin stained. x 80

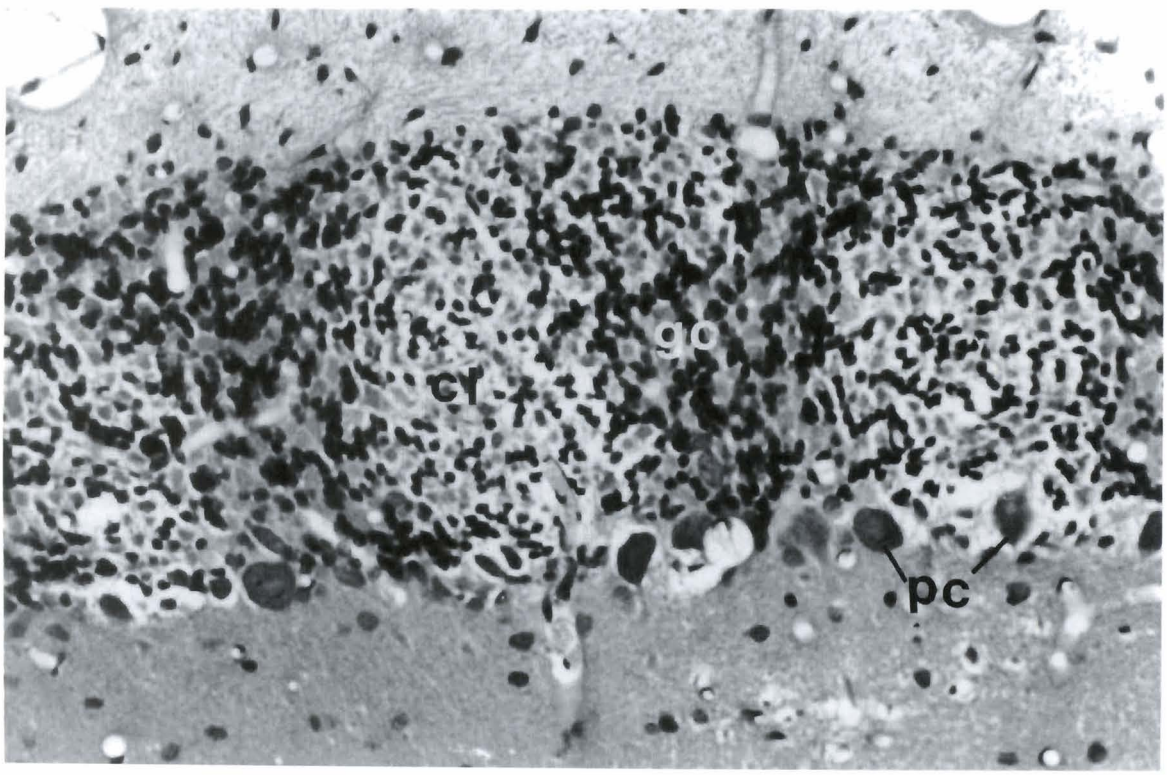

Fig. 4. Cerebellum of a 28 day old rat irradiated with 0.5 Gy on GD-17 illustrating the clusters of lesions among the normal population of granule cells within the inner granular layer. Note that within these lesioned areas, the granule cells are reduced in number and some appeared atrophied. Coronal section. PC: Purkinje cells; GC: granule cells; CL: circumscribed lesion. A higher magnification of Figure 2. Thionin stained. x 80

Purkinje cells were readily identified based on their size, flask shape, and their dendrites oriented towards the pial surface. In all irradiated groups, the Purkinje cells were aligned in a monolayer throughout the entire anterior and posterior lobes of the cerebellum.

These CL distributed within the normal granule cell population varied in size and distribution and in some instances would span across the entire width of the inner granular layer. These radiation induced lesions were found in most irradiated groups in varying proportions. Some control animals also exhibited these lesions, but the proportion was fewer, and compared to the irradiated group statistically not significant.

The incidence of cerebellar lesions within the inner granular layer is summarized in (Tables 1, 2). The values are compared using Fisher's exact test. In the cerebella of most irradiated rats the occurrence of the lesions was significantly different ($p<0.05$, Table 1) from controls. These radiation induced lesions were found in various proportions throughout the irradiated gorups. In 21 day old rats, these CL found in 78% on GD-18 and 57% on GD-19 differed significantly ($p<0.05$) from controls (Table 1). In 28 day old postnatal rats, these CL found in 83% on GD-17, 89% on GD-18, 100% on GD-19 and 80% on GD-20 differed significantly ($p<0.05$) from controls (Table 1). Irradiation within this four day period resulted in a tendency of these lesions to vary on the different days of exposure to irradiation. However, comparing the animals irradiated on GD-17 with those on GD-20, or between 21 and 28 days of age, resulted in

Table 1. Incidence of cerebellar lesions within the internal granular layer in 21- and 28-day old control and irradiated rats.

GESTATIONAL DAY OF IRRADIATION	RAT AGE DAYS	CONTROL RATS WITH LESIONS	NUMBER OF IRRADIATED RATS WITH LESIONS
17	21	3/7 (43%)	3/4 (75%)
	28	2/7 (29%)	5/6 (83%)*
18	21	1/6 (17%)	7/9 (78%)*
	28	0/7 (0%)	8/9 (89%)*
19	21	0/7 (0%)	4/7 (57%)*
	28	4/10 (40%)	10/10 (100%)*
20	21	4/9 (44%)	3/3 (100%)
	28	3/12 (25%)	8/10 (80%)*

*: $p<0.05$ significantly different from controls (Fisher's exact test).

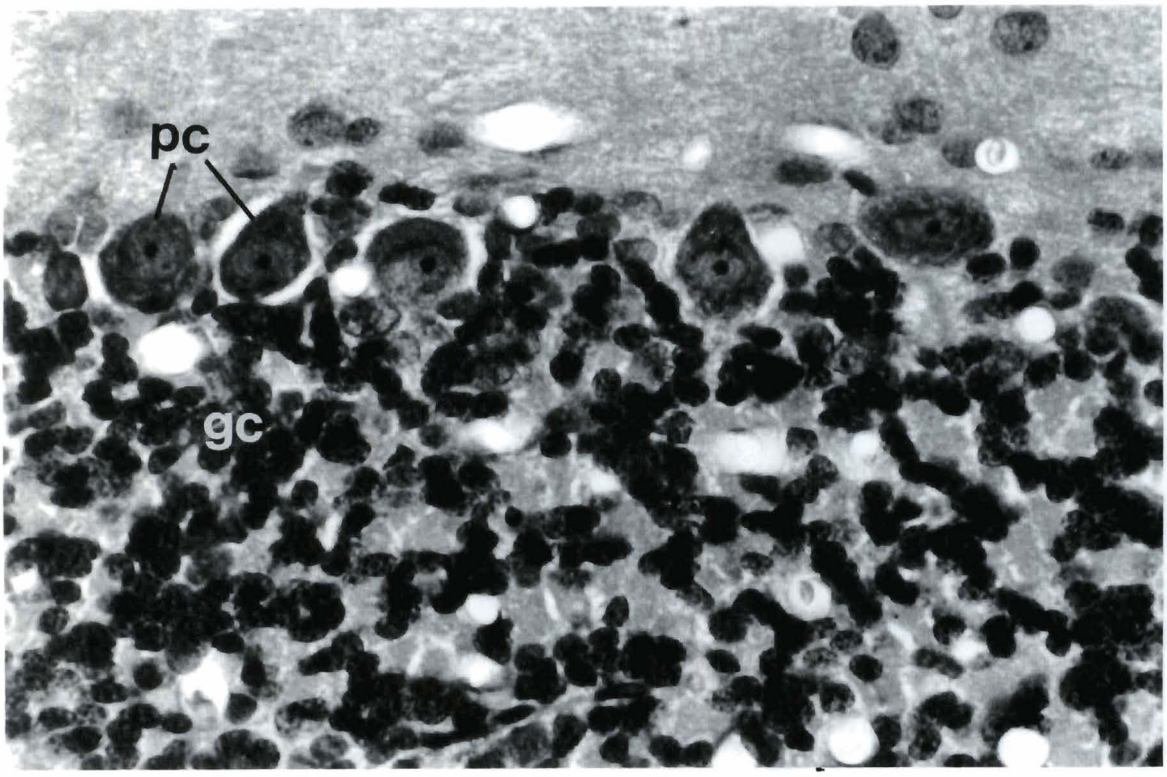

Fig. 5. Inner granular layer from the cerebellum of a 28 day old non-irradiated rat illustrating the normal population of granule cells clustered beneath the Purkinje cells. Coronal section. GC: granule cells; PC: Purkinje cells. A higher magnification of Figure 1. Thionin stained. x 160

no significant difference.

These lesions were distributed in both the anterior and posterior lobes. However, irradiation on GD-17 yielded more rats with lesions within the anterior lobe (75%) than those irradiated on GD-20 (67%). Comparing 21 day old rats irradiated on GD-17 with those on GD-20, there was a trend exhibiting more lesions within the anterior lobe (75%) on GD-17 and (67%) on GD-20. In 28 day old rats, the reverse was true. Irradiation on GD-17 yielded fewer lesions (67%), within the anterior lobe and more on GD-20 (80%) (Table 2). In the posterior lobe the only significant difference between treatment and day of irradiation was noted in 21 day old rats. Irradiation on GD-17 yielded no lesions whereas, on GD-20, all rats (100%) exhibited these lesions which differed significantly ($p<0.05$) from the controls (Table 2).

The total number of these lesions were counted

Table 2. Incidence of lesions within the internal granular layer of specific lobes of the cerebellum in 21- and 28-day old irradiated rats.

GESTATIONAL DAY OF IRRADIATION	RAT AGE DAYS	NUMBER OF RATS WITH LESIONS IN: ANTERIOR LOBE	POSTERIOR LOBE	COMBINED LESIONS (ANTERIOR AND POSTERIOR) LOBES
17	21 (4)	3 (75%)	0 (0%)	0 (0%)
	28 (6)	4 (67%)	4 (67%)	3 (50%)
18	21 (9)	7 (78%)	4 (44%)	4 (44%)
	28 (9)	7 (78%)	7 (78%)	6 (67%)
19	21 (7)	4 (57%)	2 (29%)	2 (29%)
	28 (10)	9 (90%)	9 (90%)*	8 (80%)*
20	21 (3)	2 (67%)	3 (100%)**	2 (67%)
	28 (10)	8 (80%)	8 (80%)	8 (80%)

*: $p<0.05$, significantly different between 21 days of age for the same day of irradiation (ANOVA), $F=6.72$ ($df=1$; $p=0.0125$); **: significantly different from GD-17 at 21 days of age, $F=9.23$ ($df=1$; $p=0.0038$). Number of animals are in parentheses.

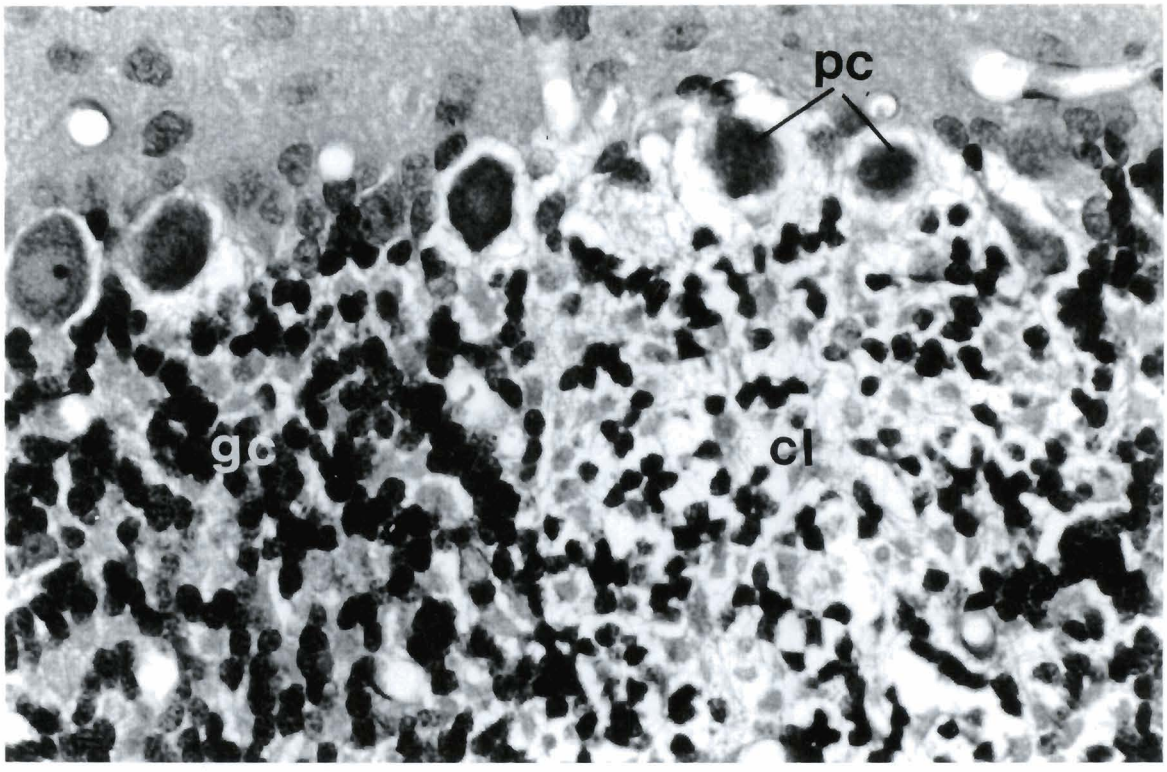

Fig. 6. Inner granular layer from the cerebellum of a 28 day old rat irradiated with 0.5 Gy on GD-17 illustrating the granule cells in a lesioned area immediately beneath the layer of Purkinje cells. Within these lesioned areas, the granule cells are reduced in number and some appear atrophied. Note the Purkinje cells immediately above the lesion are either absent or pyknotic. Coronal section. PC: Purkinje cells; GC: granule cells; CL: circumscribed lesion. A higher magnification of Figure 2. Thionin stained. x 160

Table 3. Comparison of the incidence of cerebellar lesions in the internal granular layer of the vermis and lateral hemispheres (LH) of the anterior and posterior lobes in 21- and 28-day old late prenatally irradiated rats.

GESTATIONAL DAY OF IRRADIATION	RAT AGE DAYS	ANTERIOR LOBE			POSTERIOR LOBE		
		VERMIS	LH	LESIONS IN BOTH	VERMIS	LH	LESIONS IN BOTH
17	21 (4)	2 (50%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	28 (6)	2 (33%)	4 (67%)	2 (33%)	3 (50%)	2 (33%)	1 (17%)
18	21 (9)	5 (56%)	4 (44%)	2 (22%)	1 (11%)	4 (44%)	1 (11%)
	28 (9)	5 (56%)	5 (56%)	3 (33%)	6 (67%)*	7 (78%)	6 (67%)*
19	21 (7)	2 (29%)	3 (43%)	1 (14%)	2 (29%)	2 (29%)	2 (29%)
	28 (10)	6 (60%)	9 (90%)	6 (60%)	8 (80%)*	8 (80%)*	7 (70%)
20	21 (3)	2 (67%)	1 (33%)	1 (33%)	1 (33%)	3 (100%)**	1 (33%)
	28 (10)	4 (40%)	7 (70%)	3 (30%)	6 (60%)	4 (40%)	3 (30%)

LH: lateral hemispheres. *: $p<0.05$, significantly different between 21 days of age for same day irradiation (ANOVA), $F=6.72$ ($df=1$; $p=0.0125$); **: $p<0.05$, significantly different between GD-17 at 21 days of age, $F=4.47$ ($df=1$; $p=0.0394$). Number of animals are in parenthesis.

Table 4. Comparison of the total number of cerebellar lesions in the internal granular layer of the vermis and lateral hemispheres (LH) of the anterior and posterior lobes in 21- and 28 day old irradiated rats.

GESTATIONAL DAY OF IRRADIATION	RAT AGE DAYS	ANTERIOR LOBE	POSTERIOR LOBE	COMBINED LESIONS (ANTERIOR AND POSTERIOR) LOBES
17	21 (4)	3	0	3
	28 (6)	64	33*	97
18	21 (9)	24	22	46
	28 (9)	32	68*	100
19	21 (7)	14	15	29
	28 (10)	131	144*	275
20	21 (3)	7	6	13
	28 (10)	23	45*	68

*: $p<0.05$, significantly different between 21 days for the same day of irradiation (ANOVA). Number of animals are in parentheses.

Table 5. Comparison of the total number of cerebellar lesions in the internal granular layer of the vermis and lateral hemispheres (LH) of the anterior and posterior lobes in 21- and 28-day old irradiated rats.

GESTATIONAL DAY OF IRRADIATION	RAT AGE DAYS	ANTERIOR LOBE			POSTERIOR LOBE		
		VERMIS	LH	TOTAL	VERMIS	LH	TOTAL
17	21 (4)	2	1	3	0	0	0
	28 (6)	17	47	64	14	19	33
18	21 (9)	13	11	24	2	20	22
	28 (9)	14	18	32	20	48	68
19	21 (7)	6	8	14	9	6	15
	28 (10)	50	81	131	55	89	144
20	21 (3)	5	2	7	1	5	6
	28 (10)	6	17	23	32	13	45

LH: lateral hemispheres. Number of animals in parentheses (ANOVA).

within the anterior and posterior lobes and the values compared using ANOVA. The results are summarized in Table 4. It was observed in rats irradiated on GD-17 that the total number of lesions were higher within the anterior lobe whereas on GD-20 there were more within the posterior lobe. Within the posterior lobe, there was a significant difference ($p<0.05$) with respect to the total number of lesions and the age of animals ($p<0.05$) for each day of irradiation (Table 4).

The incidence and total number of these CL within the vermis and lateral hemispheres were analyzed with ANOVA and the results are summarized in Tables 3 and 4. Within the anterior lobe, irradiation on both GD-17 and GD-20 yielded more lesions in the vermis in 21 day

Table 6. Incidence of cerebellar lesions within the internal granular layer in specified lobules in the posterior cerebellum in 21- and 28-day old irradiated rats.

GESTATIONA DAY OF IRRADIATION	RAT AGE DAYS	7 FOLIUM TUBER AND VERMIS	8 PYRAMIS	9 UVULA
17	21 (4)	0 (0%)	0 (0%)	0 (0%)
	28 (6)	2 (33%)	1 (17%)	2 (33%)
18	21 (9)	3 (33%)	2 (22%)	3 (33%)
	28 (9)	7 (78%)	6 (67%)	5 (56%)
19	21 (7)	2 (29%)	1 (14%)	0 (0%)
	28 (10)	7 (70%)	7 (70%)*	6 (60%)*
20	21 (3)	0 (0%)	3 (100%)**	1 (33%)
	28 (10)	5 (50%)	4 (40%)	4 (40%)

*: $p<0.05$, significantly different between 21 days of age for the same day of irradiation (ANOVA), $F=4.27$ ($df=1$; $p=0.0440$); **: $p<0.05$, significantly different from GD-17 at 21 days of age. Number of animals are in parentheses.

Table 7. Incidence of cerebellar lesions within the internal granular layer of the vermis and lateral hemispheres (LH) in specified lobules of the posterior cerebellum in 21- and 28-day old irradiated rats.

GESTATIONAL DAY OF IRRADIATION	RAT AGE DAYS	LOBULES					
		7 FOLIUM TUBER AND VERMIS		8 PYRAMIS		9 UVULA	
		Vermis	LH	Vermis	LH	Vermis	LH
17	21 (4)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	28 (6)	1 (17%)	2 (33%)	1 (17%)	1 (17%)	2 (33%)	0 (0%)
18	21 (9)	1 (11%)	2 (22%)	0 (0%)	2 (22%)	1 (11%)	2 (22%)
	28 (9)	6 (67%)*	6 (67%)*	3 (33%)	6 (67%)	1 (11%)	4 (44%)
19	21 (7)	2 (29%)	2 (29%)	1 (14%)	1 (14%)	0 (0%)	0 (0%)
	28 (10)	7 (70%)	6 (60%)	3 (30%)	5 (50%)	6 (60%)*	3 (30%)
20	21 (3)	0 (0%)	0 (0%)	1 (33%)	2 (67%)	0 (0%)	1 (33%)
	28 (10)	2 (20%)	3 (30%)	2 (20%)	2 (20%)	4 (40%)	0 (0%)

LH: lateral hemispheres. *: $p<0.05$, significantly different between 21 days of age for the same day of iradiation (ANOVA), $F=7.56$ ($df=1$; $p=0.0083$). Number of animals are in parenthesis.

old rats, whereas in 28 day old rats, more lesions were found in the lateral hemispheres. This difference was not statistically significant. Within the posterior lobe, irradiation on GD-18 and GD-19 produced more lesions in the vermis in 28 day old rats which was significantly different ($p<0.05$) from lesions in 21 day old rats (Table 3). Irradiation on GD-19 produced more lesions in the lateral hemispheres in 28 day old rats which was significantly different ($p<0.05$) from lesions in 21 day old rats. Within the posterior lobe, irradiation yielded more CL on GD-20 (100%) which was significantly different ($p<0.05$) from lesions in GD-17 (0%) (Table 3). The total number of CL within the anterior and posterior lobes was not statistically significant, and no interaction with day of treatment or age was observed. It was observed in 28 day old rats irradiated on GD-17 that the total number of CL was more in the lateral hemispheres of both anterior and posterior lobes whereas, on GD-20, more were found in the vermis in the posterior lobe and in the lateral hemispheres in the anterior lobe (Table 5).

The incidence of lesions in posterior lobules 7, 8, and 9 were counted and analyzed with ANOVA and the results are summarized in Table 6. In lobules, 8 and 9, irradiation on GD-19 yielded more lesions in 28 day old rats which was significantly different ($p<0.05$) from 21 day old rats. In lobule 8, in 21 day old rats, there were fewer lesions after irradiation on GD-17 (0%) which differed significantly ($p<0.05$) from GD-20 (100%). No other significant differences were observed between the lobules. The total number of lesions in lobules 7 and 9 was significantly different ($p<0.05$) between 21 and 28 day old rats for each gestational day of irradiation. In lobule 8, this same difference was almost significant ($p=0.057$) (Table 8). The incidence and total number of CL within the vermis and lateral hemispheres in lobules 7, 8 and 9 were compared using ANOVA and the results are summarized in tables 7-9. In lobule 8, there were fewer lesions with irradiation on GD-17 and more on GD-20. This was not statistically significant. No other interaction was observed. With irradiation on GD-18 there were more lesions in the lateral hemispheres and the vermis in the 28 day old rats in lobule 7 ($p<0.05$)

Table 8. Comparison of the total number of lesions within the internal granular layer in specified lobules of the posterior cerebellum in 21- and 28-day old late prenatally irradiated rats.

GESTATIONAL DAY OF IRRADIATIN	RAT AGE DAYS	7 FOLIUM TUBER AND VERMIS	8 PYRAMIS	9 UVULA
17	21 (4)	0	0	0
	28 (6)	13	16	3
18	21 (9)	5	7	6
	28 (9)	24	12	5
19	21 (7)	7	2	0
	28 (10)	49	33	27
20	21 (3)	0	3	2
	28 (10)	10	12	15

Number of animals are in parentheses (ANOVA).

Table 9. Comparison of the total number of lesions within the internal granular layer of the vermis and lateral hemispheres (LH) in specified lobules of the posterior cerebellum in 21- and 28-day old irradiated rats.

GESTATIONAL DAY OF IRRADIATION	RAT AGE DAYS	7 FOLIUM TUBER AND VERMIS		8 PYRAMIS		9 UVULA	
		Vermis	LH	Vermis	LH	Vermis	LH
17	21 (4)	0	0	0	0	0	0
	28 (6)	4	9	6	10	3	0
18	21 (9)	1	4	0	7	1	5
	28 (9)	13	11	3	9	1	4
19	21 (7)	3	4	1	1	0	0
	28 (10)	20	29	13	20	12	15
20	21 (3)	0	0	1	2	0	2
	28 (10)	7	3	8	4	15	0

LH: lateral hemispheres. Number of animals are in parentheses (ANOVA).

than in 21 day old rats. In lobule 8 the 28 day old rats had more lesions in the vermis with irradiation on GD-19 ($p<0.05$) than in the 21 day old rats. No other interaction was significant. It was observed after irradiation on GD-17 and GD-20 that more CL were found in the lateral hemispheres in lobules 7 and 8, and in lobule 9 more CL were found in the vermis (Table 7). Comparing the total number of lesions within the lobules, it was observed in lobules 7 and 8 in 28 day old rats, that irradiation on GD-17 yielded more lesions in the lateral hemispheres whereas, on GD-20, more lesions were found in the vermis (Table 9). In lobule 9, irradiation on both GD-17 and GD-20 yielded more lesions in the vermis. These differences were not statistically significant and no other interactions were noted.

Discussion

In this study, maternal exposure to a single dose of whole body irradiation (0.5 Gy) on GD-17, 18, 19 and 20 did not result in any gross cerebellar anomalies. The cerebella of all irradiated animals had attained a normal trilaminar architecture consisting of an outer molecular layer, a middle monolayer of Purkinje cells, and an inner granular layer. However, the most pronounced radiation induced change in the cerebellum was observed in the inner granular layer. Regardless of the gestational day of irradiation, all irradiated animals exhibited well defined clusters of CL distributed throughout the inner granular layer in both the anterior and posterior lobes. These lesions were characterized by a reduced number of granule cells and atrophy of some of the remaining granule cells. The Purkinje cells immediately above the lesion were either missing, atrophied or normal in appearance.

Irradiation on GD-17 resulted in more CL in the anterior lobe and vermis, whereas irradiation on GD-20 resulted in more CL in the posterior lobe and in the lateral hemispheres. These lesions coincide with a greater decrease in both Purkinje cells and granule cells in the vermis with irradiation on GD-17, whereas, with irradiation on GD-20, both of these cells were reduced in the lateral hemispheres (Ralcewicz and Persaud, 1994). It is known that Purkinje cells have a role in the induction of the development and maturation of granule cells. The number of Purkinje cells and the time these cells spend in the presence of granule cells during their

critical period of development determine the number of granule cells. Fewer Purkinje cells and inadequate interaction between Purkinje cells and granule cells result in granule cell death (Wetts and Herrup, 1983).

The difference in the location of these CL suggests developmental differences. Because the anterior lobe and vermis arises embryologically first (Larsell, 1952), the Purkinje cells and granule cells in these areas would be more sensitive to the effects of irradiation with exposure on GD-17 than on GD-20. This would result in fewer Purkinje cells and ultimately fewer granule cells. The reduction in the number of granule cells would be evident by the presence of more CL within the inner granular layer in the anterior lobe and vermis. The posterior lobe and the lateral hemispheres, which are the lateral extensions of the vermian segments, arise later, and the number of both Purkinje cells and granule cells would be more reduced with irradiation on GD-20 than on GD-17. This would result in more CL in the posterior lobe and within the lateral hemispheres with irradiation on GD-20. The medial to lateral and anterior to posterior gradient in the deficits of both granule cells and Purkinje cells observed in this radiation study has also been reported in mutant mice (Herrup and Mullen, 1979a; Chen and Hillman, 1989).

In a study by Bruni et al. (1993) rats irradiated with 0.5 Gy on GD-15 and GD-18 also exhibited similar lesions as in the present study. It was reported that these lesions were more profound with irradiation on GD-18 than on GD-15. However, the specific distribution of these lesions was not investigated.

Purkinje cells originate prenatally between embryonic days 13 to 16 and mature postnatally (Das and Nornes, 1972; Altman and Bayer, 1978a). The granule cells arise postnatally from day 4 through day 21 with the bulk being formed during the second week (Altman and Das, 1965). The questions that arise are: 1) why do these radiation induced lesions appear in the inner granule layer when the animals were irradiated at a time during which the granule cells are not being formed? 2) why are these CL arranged in clusters? Of interest, similar circumscribed lesions were found in some control animals but the proportion was relatively fewer, and compared to the irradiated group statistically not significant.

One explanation could lie in the fact that irradiation does not affect the population of granule cells directly, but rather indirectly. The reduction in the number of granule cells and atrophy of the remaining granule cells could be an indirect consequence of reduced critical interaction with Purkinje cells. The question arises what possible mechanisms are regulating the ratio of neuronal numbers between target cells, the Purkinje cells, and their afferents, the granule cells?

The results from this study can be interpreted by the following possible mechanisms. Firstly, there could be more than one class of Purkinje cells that are being affected by irradiation. It has been demonstrated in the mutant lurcher mouse, that the cerebellar cortex contains at least two classes of Purkinje cells (Tano et al., 1992). These classes of Purkinje cells are organized into alternating arrays of parasagittal bands. The classification of Purkinje cells into parasagittal bands is evident in the vermis and less in the lateral hemispheres (Hawkes and Gravel, 1991). The compartmentation of the cerebellum was demonstrated using the molecular marker zebrin, which is expressed selectively by Purkinje cells (Hawkes and Gravel, 1991; Tano et al., 1992). It has been reported for both in the vermis and lateral hemisphere, that zones of Purkinje cells, which are zebrin positive, alternate with zones that are zebrin negative (Hawkes and Gravel, 1991).

In the lurcher mouse zebrin is expressed by Purkinje cells in the pyramis, uvula and flocculonodular (Tano et al., 1992). Irradiation on GD-20 could have affected one class of Purkinje cells in the vermis that were being expressed in one zone, rendering them more radiosensitive, while the other subset of Purkinje cells were not expressed in that zone, and therefore more radioresistant to irradiation. Fewer or malformed Purkinje cells would subsequently alter the induction of granule cell development resulting in fewer granule cells. The expression of Purkinje cells in parasagittal bands could explain why the presence of the CL within the inner granular layer of the vermis is arranged in alternating clusters.

A second possible mechanism could be that irradiation altered the genetic expression of Purkinje cells which would ultimately alter their inductive control over granule cells resulting in secondary death of granule cells and the appearance of these CL within the inner granular layer. Genetic control is a major factor in the development and maintenance of mature phenotypes of neurons. Studies of genetic mutant mouse strains and exposure to prolonged X-irradiation have shown that the number and maintenance of Purkinje cells are genetically controlled (Altman and Anderson, 1972; Rakic and Sidman, 1973; Sotelo, 1975; Herrup and Mullen, 1979b; Herrup and Sunter, 1986). The genetic induced cell death of Purkinje cells in the lurcherer and staggerer results in an indirect cell death of granule cells. The loss of granule cells matches the number of Purkinje cells lost so that a constant ratio between these two types of cells is maintained (Caddy and Biscoe, 1979; Herrup and Mullen, 1979b; Wetts and Herrup, 1982a,b,c; Herrup and Sunter, 1986). Although lurcher mice are born with a normal number of Purkinje cells, these cells begin to degenerate during the beginnings of synaptogenesis. The death of granule cells follows the degeneration of Purkinje cells. In the staggerer, the Purkinje cells are genetically altered and the death of granule cells correlates with the inability of the granule cells to establish synaptic contact with the Purkinje cells. Any reduction or alteration in the number of target neurons proportionally reduces their afferents.

In the rat, Purkinje cells complete their final cell division between embryonic days 12 and 16 (Altman, 1972b; Schultze et al., 1974). Since the commitment of

Purkinje cells is already established prior to their formation from the transitory zone between embryonic days 13 to 16 (Altman, 1972b; Das and Nornes, 1972; Schultze et al., 1974; Altman and Bayer, 1978a), irradiation between GD-17, 18, 19 and 20 could not be affecting the progenitors of Purkinje cells. Irradiation between GD-17 and GD-20 coincides with the stage of differentiation and migration of Purkinje cells. Irradiation during this time could alter the genetic expression of some but not all of the Purkinje cells. On embryonic day 14 to 15, the early Purkinje cells begin to differentiate and, by embryonic day 16 to 17, these cells begin to migrate radially from the transitory zone through the differentiating neurons in the nuclear zone and cluster superficially underneath the intermediate fibrous layer (Altman and Bayer, 1978a). Depending on when the Purkinje cells arise, the migration and dispersion occurs up to approximately four days after birth (Altman, 1972b).

Irradiation could also alter the synthesis of proteins required to produce the exogenous factors or signals for the induction of granule cells by Purkinje cells. Alternately, irradiation between GD-17 and GD-20 could interfere with the synthesis of the neural-glia adhesion molecule astrotactin which is required for the positioning and cell specification of granule cells onto the radial glial fiber.

The glycoprotein astrotactin functions as a ligand which binds the migrating neuron to the glial fiber (Edmondson et al., 1988; Hatten and Mason, 1990; Stitt and Hatten, 1990). It has been reported that antibodies against astrotactin prevents the neuron from binding to the glial fibers (Stitt and Hatten, 1990). The binding of the neuron to the glial cell is required to establish the neuron-glia contact and to organize the position of the neuron onto the glial fiber (Edmondson et al., 1988). Astrotactin is expressed in granule neurons at specific stages of development. It is abundant in postnatal cells and in migrating neurons (Hatten and Mason, 1990). Astrotactin is expressed in high levels during granule cell migration along radial glial fibers and during assembly of the granule cells into the inner granular layer (Stitt and Hatten, 1990). Any alteration in the expression of astrotactin prior to granule cell migration could alter both the neuron-glial contacts and the organization and positioning of the granule neuron onto the glial fiber. This would ultimately result in fewer granule cells assembling into the inner granular layer and would be manifested by the presence of radiation induced lesions within the inner granular layer. Another possible mechanism could be that irradiation may be turning on the genetic expression of programmed cell death. This could explain why a certain percentage of control animals also exhibited these CL, although not statistically significant, within the inner granular layer. It is known that natural cell death is a principal mechanism which regulates the overproduction and selective interaction between target cells and their afferents (Cunningham, 1982; Cowan et al., 1984; Oppenheim, 1985).

Physiological cell death is a normal developmental process observed in vertebrates and invertebrates (Clarke, 1990; Vaux, 1993). Cells undergoing natural cell death exhibit changes termed PCD-programmed cell death or apoptosis (Vaux, 1993). Apoptosis allows for the elimination of damaged, excessive or precancerous cells (Schulte-Hermann et al., 1992). The characteristic changes accompanying cells undergoing apoptosis include rapid DNA fragmentation of isolated cells with no inflammatory infiltrate (Clarke, 1990; Vaux, 1993). Multicellular organisms have developed the molecular mechanisms to implement cell death. The occurrence of cell death is widespread and is observed during the development of the gastrointestinal tract (Potten, 1992) during insect metamorphosis (Lockshin, 1969), retinal development in humans (Provis and Van Driel, 1985), and regression of Müllerian and Wolffian ducts in sexual development (Ortiz, 1945). Naturally occurring cell death has an important role in controlling the size, shape and constitution of compartments in normal developmental processes (Gavrieli et al., 1992; Oren, 1992). Cell death also exerts a homeostatic function in maintaining a balance between cell replication and cell death (Gavrieli et al., 1992). It is known that cell death exerts a protective role against carcinogenesis and disease (Schulte-Hermann et al., 1992). This is evident in the removal of skin cells during exposure to UV radiation (Danno and Horio, 1982) and in the removal of epithelial cells in the gastrointestinal tract during exposure to carcinogens in the diet (Potten et al., 1977). Programmed cell death can also be induced by manipulating the hormone levels in hormone dependent tissues such as the prostate and the mammary gland (Tenniswood et al., 1992) and by radiation (Potten, 1992). Potten (1992) had reported that cell death in the crypts of the gastro-intestinal mucosa was elevated by small exposure radiation with doses of 1, 9, and 12 Gy. There was a strong dose-response dependence from 0 to 1 Gy.

The lesions occurring within the inner granular layer in control animals could be granule cells undergoing the natural process of cell death, to remove the excess granule cells produced during development, as a part of maintaining homeostatic function. In the animals irradiated on each of GD-17, 18, 19 and 20 there was a significant high proportion of these lesions observed in the inner granular layer. These lesions coincided with a loss in the number of granule cells and atrophy of some of the remaining granule cells. Radiation between GD-17 and GD-20 could have induced spontaneous cell death in attempts to eliminate the granule cells that were genetically affected during exposure to radiation. By eliminating the unwanted defective granule cells, the overall homeostatic balance is maintained. Programmed cell death does not occur randomly but rather preferentially (Schulte-Hermann et al., 1992). Gavrieli et al. (1992) has reported that PCD appears in tissues in clusters. The lesions appearing in the inner granular

layer in this study occurred in clusters and is in agreement with Gavrieli et al. (1992).

Another possible explanation for the appearance of these lesions within the inner granular layer is that irradiation could have affected the precursors of granule cells that were replicating simultaneously as the Purkinje cells, thereby, altering the genesis of granule cells.

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. Furthermore, this study supports the view that both cell death and the regulation of granule cells by Purkinje cells play an essential role in the effective development and organization of the cerebellum.

Acknowledgements. The authors thank the Unsolicited Proposals Fund, Department of Supply and Services; Atomic Energy Control Board and Health and Welfare, Canada for funding this investigation. We also thank Dr. Paul Wong (NIH) for his valuable suggestions, and Mr. R. Simpson and Mr. G. Seyoum for their technical assistance.

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Accepted November 30, 1994