

LONG-TERM CHANGES IN NORADRENALINE (NA) AND DOPAMINE (DA) CONTENTS OF RAT CEREBELLUM FOLLOWING NEONATAL X-IRRADIATION

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Abstract—The effects of neonatal X-irradiation on cerebellar catecholamine levels in rats were studied at different postnatal intervals. Since synaptogenesis in the cerebellar cortex is basically a postnatal phenomenon, changes in noradrenaline (NA) and dopamine (DA) levels induced by X-rays on the cerebella (CE) of adult rats (60 days old) were also studied. With 200 rad at birth there was an increase in both NA (+75%) and DA (+40%) levels at day 30, with a return to control values by day 90. CE weight did not change with this dose. Both 500 and 700 rad given at birth induced a persistent increase in NA levels, even when studied at day 390 (+127%) and a long-term decrease in DA levels. A marked atrophy of CE was found, even at day 390 (a 61% decrease in weight). Histologic analysis showed that the cerebellar cortex lacked its interneurons (agranular cerebellar cortex) and that Purkinje cells were randomly arranged. Rats showed dystonia, fine tremor, posterior train ataxia and microcephalia.

On the other hand, X-irradiation of adult rats did not change cerebellar catecholamine levels or produced cerebellar atrophy. These animals did not show motor deficits or microcephalia.

Taken together, these results suggest that the long-term changes in cerebellar catecholamine levels induced by neonatal X-irradiation may be somehow related to the loss of cerebellar interneurons which develop early in the postnatal period, although a primary change(s) in the activity of noradrenergic neurons can not be excluded.

The cerebellum of rodents provides a useful model to study the phenomena related to synaptogenesis. During maturation of the nervous system the final accuracy of synaptic contacts is achieved only after remodeling of connections established at earlier developmental stages and elimination of redundant synapses is one of the mechanisms which determines the adult connectivity (Changeux and Danchin, 1976; Huttenlocher *et al.*, 1982; Lierse, 1986). Synaptogenesis in the cerebellar cortex is basically a postnatal process. The “regression” of redundant synaptic connections that are formed during maturation can be prevented by the selective destruction of the matrix granule cells of rats whose cerebella were repeatedly X-irradiated from birth (Altman, 1971 a,b; Kameyama and Hoshino, 1986; Lierse, 1986).

The fibers entering the cerebellar cortex that are called, respectively, climbing, mossy and monoaminergic, interact with the developing cellular matrix. The fibers that contain noradrenaline (NA), are the first axons to invade the primitive cerebellum as early as days 16–17 of gestation. At birth, they run diffusely within the primordium of the granular layer and the Purkinje cell layer, with a few fibers being found in the external granular layer (Berry *et al.*, 1981).

The noradrenergic innervation of the cerebellar cortex can be modified by neurotoxic compounds. The neonatal injection of 6-hydroxydopamine or its precursor amino acid 6-hydroxydopa produce a marked and persistent increase in cerebellar NA levels (Breese and Traylor, 1970, 1971; Jaim-Etcheverry and Zieher, 1975; Zieher and Jaim-Etcheverry, 1979). While adrenergic nerve terminals are destroyed in the forebrain, elevated levels of NA in the cerebellum may result from the accumulation

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of the amine in the undamaged portions of the neurons as well as their collateral sprouting in the vicinity of the cell bodies in response to the chemical injury (Jaim-Etcheverry and Zieher, 1975, 1979; Sachs and Jonsson, 1975; Zieher and Jaim-Etcheverry, 1979).

On the other hand, it has been shown that X-irradiation from birth to 18 days produces histological changes in cerebellar cortex such as: (a) a lack of the acquisition of the postnatally-forming basket, stellate and granule cells; (b) the somata of Purkinje cells are not strung out in a monolayer and their primary dendrites are randomly oriented; (c) Purkinje cells sprout innumerable small processes and form postsynaptic dense membranes in contiguity with inappropriate processes (Altman and Anderson, 1971, 1972; Anderson and Stromberg, 1972 a, b, c, d; Berry *et al.*, 1981; Sotelo, 1977).

Since the development of the monoaminergic innervation of the cerebellum seems to be also related to the development of the different cell types that constitute the cortex as indicated by observations done in several specific mutants (Ghetti *et al.*, 1987; McKeon *et al.*, 1986) or after different drug treatments (Jaim-Etcheverry and Zieher, 1975, 1979; Sachs and Jonsson, 1975), we considered it of interest to study the effects of a single dose of X-irradiation given at birth, instead of multiple doses as has been done by other authors, on cerebellar monoamine levels and to compare them with the changes induced by the neonatal administration of neurotoxic compounds.

Since synaptogenesis of rat cerebellar cortex is basically a postnatal phenomenon, the changes produced by X-irradiation on cerebella of adult rats were also studied. We also attempted to find a relationship between changes in cerebellar monoamine levels with the occurrence of histological and motor abnormalities.

EXPERIMENTAL PROCEDURES

Animals

Newborn littermate Wistar rats of both sexes were separated into experimental and control (non-irradiated) groups.

Radiation procedure

The radiation source was a 220 kV, 8 mA, X-ray unit (Philips 220/25 for profound radiotherapy, Philips Groeilmpen Fabrieken, Eindhoven, Netherlands). Distance between the source and the animals was 28 cm. A 0.5 Cu²⁺ 1 Al³⁺ filter equivalent to 1.3 mm Cu²⁺ half-level (HVL) was used for filtration. A Simplex probe (Simplex Universal Dosimeter Physikalisch Technische Werkstätten, Freiburg, Germany) was used for dosimetry. Exposure time to each dose (200, 500 or 700 rad) was fixed by previous dosimetry.

Animals were not anesthetized. Only the head of the animal (up to 48 h after birth) was exposed to a single dose of X-radiation, the body being protected with a 4 mm Pb³⁺ sheet (i.e. spinal cord not irradiated). Heads of each litter (8 animals) were arranged circularly in the radiation field, set on a 0.5 cm thickness wax phantom. Heads of littermates were exposed to each single dose of X-irradiation simultaneously.

X-irradiation on adult rats

Littermate Wistar rats of both sexes were separated into experimental and control (non-irradiated) groups. Animals were irradiated at day 60 of postnatal life and killed by decapitation 30 days after X-irradiation. Irradiation was carried out as described above.

Tissue processing and NA and DA determinations

Animals were killed by decapitation at 30, 90, 150, 185 or 390 days of age. The brain was exposed, the olfactory tubercles and the pineal gland were discarded and, after determining its weight, the cerebellum was homogenized in 5 ml perchloric acid (0.4 N perchloric acid, 0.2% EDTA, 0.05% Na₂S₂O₅). The homogenates were kept at 4°C for 24 h and then centrifuged at 3500 rpm for 10 min. An aliquot of the supernatant was brought to pH 8.2 with the addition of one and a half volumes of Tris buffer (pH 10.5, 0.5 M). The aliquot then was poured into a column of 5 mm internal diameter packed with 200 mg of alumina. After passing the homogenate, the column was washed with 1 ml of sodium acetate (0.2 M pH 8.2) and 2 ml of water. NA and DA were eluted with 3 ml of acetic acid 0.2 N (Adler-Graschinsky *et al.*, 1972; Graefe *et al.*, 1973). The fluorometric determinations of NA and DA were carried out according to Laverty and Taylor (1968).

Histologic analysis

Rats were anesthetized with pentobarbital (3.5 mg/100 g body wt, i.p.) and perfused through the left ventricle with a solution containing 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (freshly prepared). Before fixation, a brief wash with a solution of the following composition (NaCl 0.9% w/v; 0.5 ml NaNO₂ 0.4 M; 2500 UI heparin) was done. Brains were postfixed in 4% paraformaldehyde, phosphate buffer 0.1 M for 2 h and washed in a solution of 5% w/v sucrose, phosphate buffer 0.1 M. Blocks were included in Paraplast after dehydration in graded alcoholic solutions and cut in a Minot microtome. The thick sections (5 µm) were stained with Gallocyanine (1 h, 56°C) and mounted in Histomount for light microscopy.

Statistical analysis

The significance of differences between values was determined by means of ANOVA (Dunnet's test) (Snedecor and Cochran, 1967).

RESULTS

Figure 1A shows the evolution of amine levels in the cerebellum at several time intervals after a single dose of 200 rad X-irradiation at birth. At day 30 there was a 75% increase in NA concentration (control: 0.45 ± 0.03 µg/g; treated: 0.78 ± 0.19 µg/g) which returned to control values by day 90. Likewise,

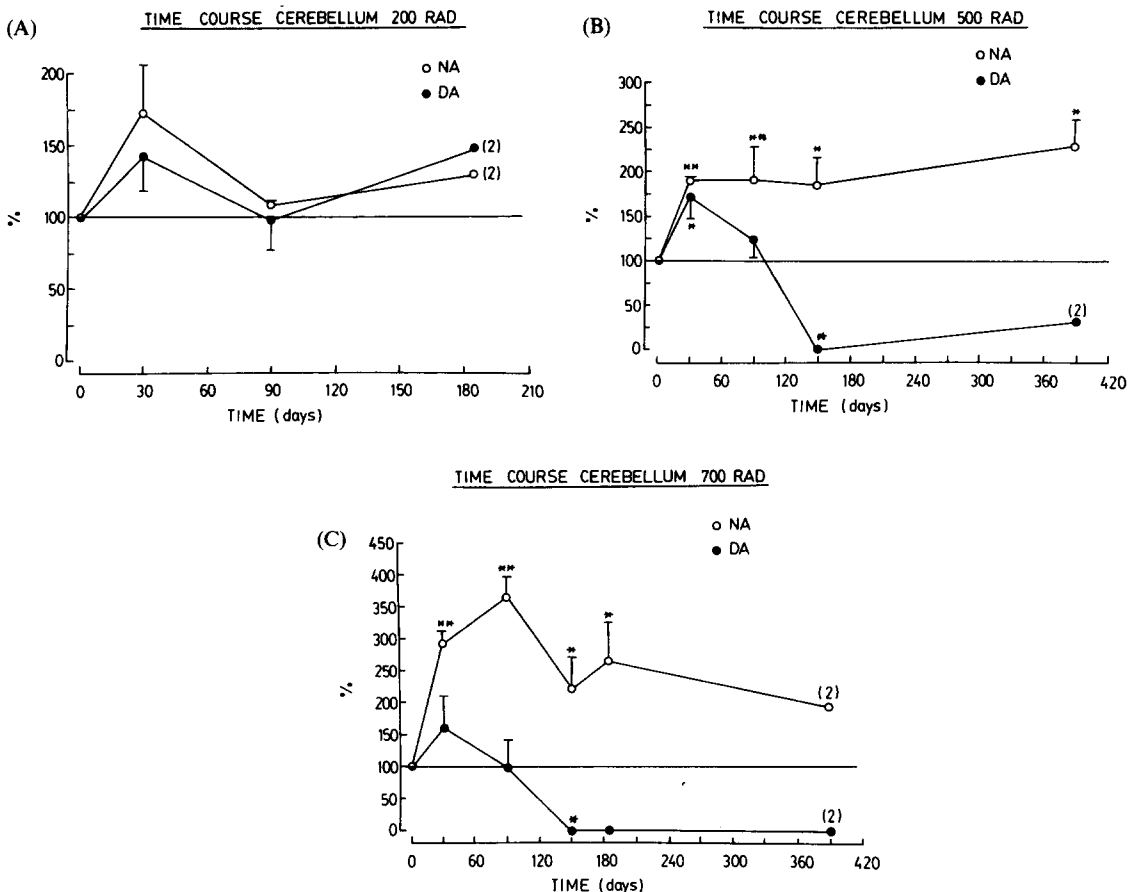


Fig. 1. Time course of modifications in the endogenous noradrenaline (NA) and dopamine (DA) concentrations of adult cerebellum induced by different doses of neonatal X-irradiation. Heads of animals were exposed to a single dose of ionizing radiation (200, 500 or 700 rad) in the neonatal period (up to 48 h from birth); animals were killed at various ages. The results are expressed as percentages of non-irradiated (control) values. Absolute control values for NA and DA ($\mu\text{g/g}$ weight) were, respectively, postnatal day (PD) 30, 0.45 ± 0.03 and 0.44 ± 0.04 ; PD 90, 0.24 ± 0.02 and 0.16 ± 0.08 ; PD 150, 0.30 ± 0.01 and 0.14 ± 0.06 ; PD 185 and 390, 0.14 ± 0.01 and 0.08 (2). Each value represents the mean \pm SEM of 3–4 determinations except when indicated between parentheses. *Significantly higher or lower levels than found in controls ($P < 0.05$). **Significantly higher or lower levels than found in controls ($P < 0.01$).

DA levels showed a 42% increase at day 30 (control: $0.44 \pm 0.04 \mu\text{g/g}$; treated: $0.62 \pm 0.12 \mu\text{g/g}$) returning to non-irradiated values by day 90 and showing a later increase at day 185.

Figure 1B shows the variations in amine levels after a single dose of 500 rad X-irradiation. NA concentration increased significantly (90%) at day 30 (control: $0.45 \pm 0.03 \mu\text{g/g}$; treated: $0.86 \pm 0.02 \mu\text{g/g}$) and remained still elevated at day 390. On the other hand, DA showed a 70% increase at day 30 (control: $0.44 \pm 0.04 \mu\text{g/g}$; treated: $0.76 \pm 0.10 \mu\text{g/g}$) but it returned to control values at day 90. Furthermore, DA levels reached non-detectable values by day 150 and

remained at very low levels at day 390 (25%).

Figure 1C shows the evolution of the variables studied after a single dose of 700 rad X-irradiation. NA concentrations increased at every interval of time taken, with a "peak" (250%) at day 90 (control: $0.24 \pm 0.02 \mu\text{g/g}$; treated: $0.87 \pm 0.07 \mu\text{g/g}$). DA levels showed an early increase of 50% at day 30 (control: $0.44 \pm 0.04 \mu\text{g/g}$; treated: $0.69 \pm 0.22 \mu\text{g/g}$) and returned to untreated values at day 90. By day 150, DA reached non-detectable values and could not be found even at day 390.

The amine content, expressed as ng/cerebellum , is shown in Tables 1, 2 and 3. There was a significant

Table 1. Effects of 200 rad X-irradiation at birth on cerebellar catecholamine contents in rats determined at different postnatal intervals.

| Postnatal day | Noradrenaline (ng) | | Dopamine (ng) | |
|---------------|--------------------|--------------|---------------|-------------|
| | C | X | C | X |
| 30 | 71.5 ± 6.7 | 116.6 ± 29.1 | 69.2 ± 7.1 | 97.7 ± 22.2 |
| 90 | 43.9 ± 4.2 | 55.2 ± 2 | 28.5 ± 13.8 | 33.1 ± 9.2 |
| 185 | 32.1 ± 5.3 | 42.2 (2) | 17.1 (2) | 28.5 (2) |

C, controls (non-irradiated); X, treated. Each value is the mean of 3–4 experiments ± SEM except when indicated between parentheses.

Table 2. Effects of 500 rad X-irradiation at birth on cerebellar catecholamine contents in rats determined at different postnatal intervals

| Postnatal day | Noradrenaline (ng) | | Dopamine (ng) | |
|---------------|--------------------|------------|---------------|-------------|
| | C | X | C | X |
| 30 | 71.5 ± 6.7 | 68.9 ± 4.6 | 69.2 ± 7.1 | 58.4 ± 9.5 |
| 90 | 43.9 ± 4.2 | 54.6 ± 3* | 28.5 ± 13.8 | 23.9 ± 0.01 |
| 150 | 30.5 ± 2.4 | 31.7 ± 1.6 | 14.5 ± 7.2 | 0* |
| 390 | 32.1 ± 5.3 | 51.21 (2) | 17.1 (2) | 5.7 (2) |

C, controls (non-irradiated); X, treated. Each value is the mean of 3–4 experiments ± SEM except when indicated between parentheses.

*Significantly higher or lower levels than found in controls ($P < 0.05$).

Table 3. Effects of 700 rad X-irradiation at birth on cerebellar catecholamine contents in rats determined at different postnatal intervals

| Postnatal day | Noradrenaline (ng) | | Dopamine (ng) | |
|---------------|--------------------|--------------|---------------|-------------|
| | C | X | C | X |
| 30 | 71.5 ± 6.7 | 75.1 ± 4.7 | 69.2 ± 7.1 | 38.7 ± 10.9 |
| 90 | 43.9 ± 4.2 | 76.0 ± 2.9** | 28.5 ± 13.8 | 13.2 ± 5.5 |
| 150 | 30.5 ± 2.4 | 28.1 ± 2.8 | 14.5 ± 7.2 | 0* |
| 185 | 32.1 ± 5.3 | 50.2 ± 5.3* | 17.1 (2) | 0 |
| 390 | 32.1 ± 5.3 | 24.1 (2) | 17.1 (2) | 0 (2) |

C, controls (non-irradiated); X, treated. Each value is the mean of 3–4 experiments ± SEM except when indicated between parentheses.

*Significantly higher or lower levels than found in controls ($P < 0.05$).

**Significantly higher or lower levels than found in controls ($P < 0.01$).

increase in total NA content of cerebellum induced by 700 X-irradiation at day 90 (+73.1%).

Figure 2 shows the time course of modifications in cerebellar weight induced by the different doses of ionizing radiation used. The lower dose (200 rad) did not change significantly cerebellar weight except for a transient increase at day 90. On the other hand, both 500 and 700 rad induced a marked decrease in cerebellar weight at day 30 (–50.2 and –73.9%, respectively); in both cases, cerebellar weight failed to return to control values even at day 390 (–21.6 and –60.9%, respectively).

Histologic findings

After a single dose of 500 rad at birth it was found that: (1) the thickness of the granular layer was markedly reduced; this resulted in an “agranular” cerebellar cortex lacking basket, stellate and late granule cells; (2) the somata of Purkinje cells were not strung out in their characteristic monolayer; (3) the primary dendrites of Purkinje cells (apical poles) were randomly oriented (Fig. 3).

Behavioral and motor deficits

Rats irradiated in the neonatal period presented dystonia, fine tremor and posterior train ataxia. There was a weakness of the hindlimbs and a tendency to fall when righting (particularly with 500 or 700 rad). Animals also exhibited microcephalia. Both the motor deficits and microcephalia reverted with time only if they were induced with 200 rad X-irradiation.

X-irradiation on adult rats

Neither 200 or 500 rad X-irradiation induced significant changes in cerebellar NA content (ng/wet whole tissue) and NA concentration ($\mu\text{g/g}$). Similar results were found for DA levels (Table 4).

Cerebellar weight did not change after ionizing treatment (C: 235 ± 1.3 mg; 200 rad: 259.3 ± 12.7 mg; 500 rad: 258.7 ± 6.7 mg). Hystologic analysis was not done in rats irradiated with this scheme.

Animals did not show motor and behavioral deficits or microcephalia.

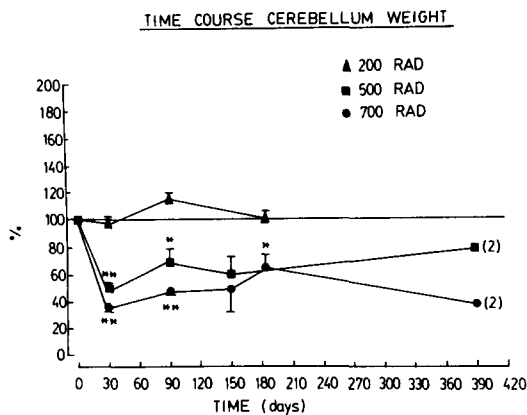


Fig. 2. Time course of modifications in cerebellum weight induced by different doses of neonatal X-irradiation. Heads of animals were exposed to a single dose of X-rays (200, 500 or 700 rad) in the neonatal period (up to 48 h from birth). Animals were killed at various ages. The results are expressed as percentages of non-irradiated control values. Each value represents the mean \pm SEM of 3-4 cerebella except when indicated between parentheses. *Significantly higher or lower levels than found in controls ($P < 0.05$). **Significantly higher or lower levels than found in controls ($P < 0.01$).

DISCUSSION

The present results show that X-irradiation of the cephalic end of neonatal rats induces long-lasting changes in cerebellar catecholamine levels, whose nature depend on the dose of radiation used. With 200 rad, there was a transient increase in NA concentration at day 30, unrelated to a decrease in cerebellar weight, that returned to control values by day 90. On the other hand, both 500 and 700 rad increased levels of NA in the cerebellum which remained abnormally elevated even at day 390. NA concentrations in the different brain structures attain adult values by 30 days without showing further changes (Loizou and Salt, 1970). Although our control values remained relatively constant from day 90, since we did not perform any determination between days 30-90, we could not establish exactly the time when adult values were reached in our animals. The changes in catecholamine levels induced by X-irradiation were also expressed as percentages of nonirradiated age-matched (control) values. A problem that complicates the study of the NA system in natural or experimental diseases of the cerebellum is that in the presence of atrophy, the NA concentration in the cerebellum is increased, whereas the NA content is not significantly changed or is even reduced. For example, in the cerebellum of the Purkinje cell

degeneration (pcd) mutant mice there is no significant change in NA content during or after Purkinje cell degeneration, but cerebellar atrophy determines a significantly increase in NA concentration (Ghetti *et al.*, 1981, 1987). Our results showed that both 500 and 700 rad given neonatally markedly decreased cerebellar weight, a reduction present even at day 390. Histological analysis showed that this atrophy reflected the lack of cerebellar interneurons (basket cells, stellate cells and late granule cells). It also showed (Fig. 3) that Purkinje cells failed to arrange in their characteristic monolayer and that they lost the normal orientation of their primary dendrite towards the molecular layer. Instead, this "apical pole" of Purkinje cells was randomly oriented. All these findings are in agreement with previous studies (Anderson and Stromberg, 1972 a, b, c, d; Sotelo, 1977) and were explained by the lack of granule cells and the disarrangement of parallel fibers in X-irradiated cerebellum at the neonatal period. Although these studies used repeated low doses of X-irradiation (150-200 rad) from birth to days 15-20, so as to prevent the postnatal development of late maturing structures, we found the same histological changes with a single higher dose (500-700 rad) immediately after birth. This suggest that this higher dose might induce a long-lasting damage even on structures that would have acquired complete development between days 15-20 of the postnatal period. For most kinds of cells radiation-induced cell death occurs when the cell is going to divide. However, cell destruction does not always occur after the first division and cells may complete one, two and even three division cycles before dying. These cells are known as "doomed cells" (Hall, 1978; United Nations, 1982). Although it was reported that a variable number of cells in the external germinal layer survive after a single dose of 150-200 rad X-rays after birth and were able to reconstitute the germinal matrix (Woodward *et al.*, 1974), this seems not to be the case in our study. Instead, a single higher dose (500-700 rad) after birth might impair the reconstitution of the germinal matrix by inducing the formation of "doomed cells".

Previous studies reported that the early postnatal developing matrix cells are very sensitive to ionizing radiation (Altman, 1971a,b; Berry *et al.*, 1981). On the contrary, a marked atrophy of the cerebellum was not found when 100 rad X-irradiation was applied to rats prenatally (postconceptual age of 15 days) (Deroo *et al.*, 1986; Inouye and Kameyama, 1983). In the early postnatal period, matrix cells actively proliferate and produce cerebellar interneurons. Since no

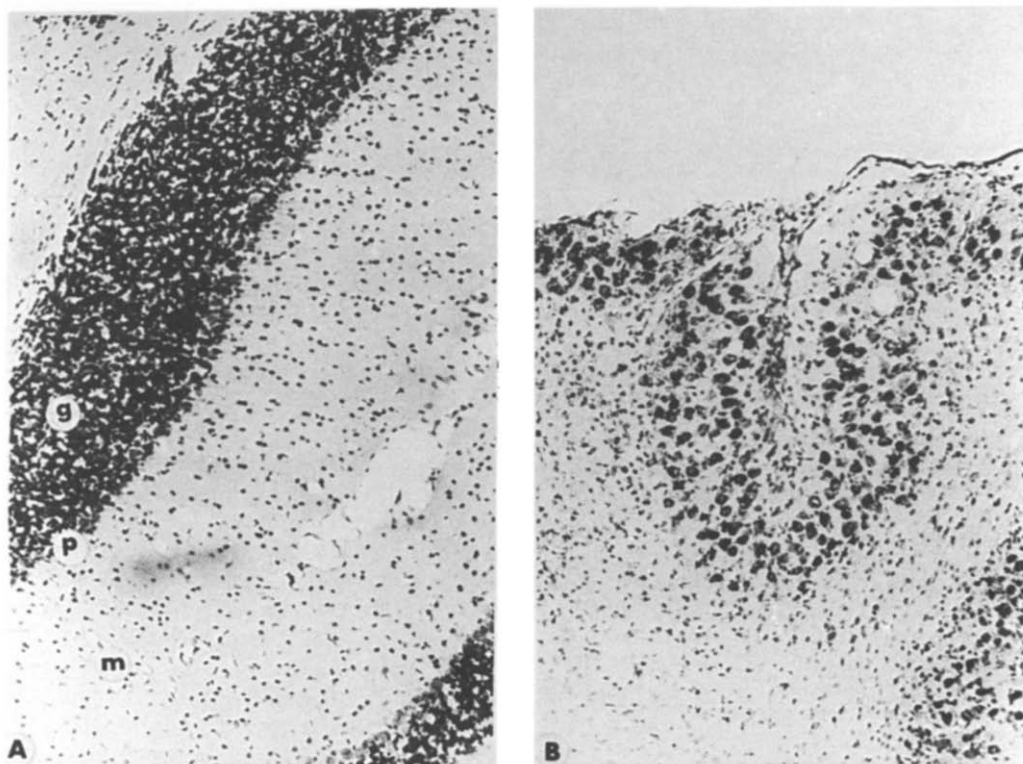


Fig. 3. Light micrographs (160 \times) showing adult cerebellar cortices of a non-irradiated control animal (A) and a 500 rad neonatally X-irradiated animal (B) stained with Gallocyanine; m: molecular layer; g: granular layer; p: Purkinje cells monolayer.

more neurons are produced after the 3rd week and the development of the cerebellum is concluded in the 4th week (Altman, 1971b; Berry *et al.*, 1981; Kameyama and Hoshino, 1986; Lierse, 1986) we also irradiated adult animals (60 days old). We could not find any change in cerebellar NA content in these animals. Likewise, they did not show neither atrophy of cerebellum nor motor abnormalities.

The increase in cerebellar concentration of NA should not be related only to cerebellar atrophy. On the contrary, increased levels of NA could also be seen when results were expressed as ng/whole

cerebellum (NA content). The great bulk of noradrenergic afferents to the cerebellum originates from NA nerve cells in the dorsolateral part of the locus coeruleus (groups A6 and A4) (Dahlstrom and Fuxe, 1964; Hoffer *et al.*, 1971; Lindvall and Bjorklund, 1974; Olsen and Fuxe, 1971; Ungerstedt, 1971). It was reported that NA fibers acquired maturity in the prenatal period of development (Hoffer *et al.*, 1971; Lierse, 1986). Furthermore, one single neuron in the dorsolateral locus coeruleus area can monosynaptically innervate both the cortex cerebri and cerebelli (Olson and Fuxe, 1971; Ungerstedt, 1971). Since we

Table 4. Effects of X-irradiation at the adult period (60 days old) on cerebellar catecholamine levels in rats determined 30 days after treatment

| | Noradrenaline | | Dopamine | |
|---------|-----------------|--------------------|-----------------|--------------------|
| | Total (ng) | Concn (μ g/g) | Total (ng) | Concn (μ g/g) |
| C | 61.8 \pm 3.2 | 0.26 \pm 0.01 | 46.3 \pm 4 | 0.20 \pm 0.02 |
| 200 rad | 69.4 \pm 10.8 | 0.27 \pm 0.05 | 29.9 (2) | 0.11 (2) |
| 500 rad | 71.5 \pm 9.2 | 0.27 \pm 0.03 | 56.4 \pm 13.3 | 0.22 \pm 0.05 |

C, controls (non-irradiated). Each value is the mean of 3–4 experiments \pm SEM except when indicated between parentheses.

did not find long-lasting changes in NA levels in the cerebral cortex after neonatal X-irradiation (Zieher *et al.*, 1987), we may speculate that the higher cerebellar levels found could be explained by a "local" phenomenon. Although we did not identify morphologically noradrenergic fibers, we cannot exclude that the higher NA levels induced by X-irradiation in the cerebellum could be related to the formation of anomalous sprouts of cell bodies as it was reported for neurotoxic compounds (Jaim-Etcheverry and Zieher, 1979, 1975; Sachs and Jonsson 1975; Zieher and Jaim-Etcheverry 1973; 1979). These sprouts might result from a direct effect of ionizing radiation or from a secondary change(s) related to the loss of cerebellar interneurons. In this way, it was reported that in the cerebellum of rats exposed to repeated doses of low level X-irradiation applied during the first two weeks of postnatal life, the innervation of single Purkinje cells by more than one climbing fiber could be found. Furthermore, it was suggested that NA terminals might appear in equal or greater than normal numbers (Freedman *et al.*, 1977; Hoffer *et al.*, 1976; Woodward *et al.*, 1974). Likewise, Landis *et al.* (1975) showed by histofluorescence that the degranulated cerebellum in mutant mice possessed a dense network of norepinephrine-containing fibers. In the pcd mutant mice, which shows similar motor abnormalities than those found in rats X-irradiated neonatally, there is evidence not only of the persistence of NA fibers following the degeneration of Purkinje and granule cells, but also of a progressive increase in the density of these fibers as shown by light and electron microscopy (Felten *et al.*, 1986; Triarhou and Ghetti, 1986).

X-irradiation damage seemed not to be, as it was shown for neurotoxic compounds, selective for neuronal elements. Instead, the lack of parallelism in the arrangement of glial fibrillary acid protein (GFAP) found in X-irradiated cerebellum (results of experiments not shown) suggests an impairment of glial cells disposition. Then, X-irradiation might also alter the normal interaction between neuronal and glial cells, an important factor in the morphogenesis of brain (Rakic, 1981; Reyners *et al.*, 1986).

The role of NA in the cerebellum is not fully understood. It has been suggested that NA might consolidate the motor signals to be stored in the cerebellum (Gilbert, 1974). Thus, cerebellar norepinephrine depletion by 6-OH-DA impaired the acquisition of specific locomotor tasks in rats (Watson and McElliott, 1984). Confirming previous reporters (Altman and Anderson, 1972; Woodward *et al.*, 1974), X-irradiated animals in the neonatal

period showed dystonia, fine tremor and posterior train ataxia. It is interesting to point out that in genetic dystonic rats, in which NA levels in cerebellum are persistently increased, reserpine failed to deplete NA stores as effectively as it did in normal rats (McKeon *et al.*, 1986). Thus, in neonatally X-irradiated and in genetic dystonic rats there is a coincidence of motor deficits and elevated levels of NA in the cerebellum.

As it was shown with NA, DA levels increased with 200, 500 and 700 rad at day 30. Although they reached control values 90 days after 200 rad, DA levels decreased and disappeared both with 500 and 700 rad by day 150. Dopamine in the cerebellum is almost exclusively localized in noradrenergic neurons. It thus represents an intermediary product (Bjorklund and Lindvall 1984; Olson and Fuxe, 1971; Ungerstedt, 1971). Since the lowest values of DA were obtained when NA levels reached their higher ones, we can speculate that X-irradiation might induce peculiar changes in the activity of noradrenergic neurons. It was reported that tyrosine hydroxylase activity was reduced both when assayed *in vitro* (Roffler-Tarlov *et al.*, 1984) and *in vivo* (Ghetti *et al.*, 1987) in (pcd) mutant mice, in which Roffler-Tarlov *et al.* (1984) have reported statistically significant increases in NA content in the cerebellar cortex. Then, it would be interesting to study tyrosine hydroxylase and dopamine beta hydroxylase activities in the cerebellum of rats after neonatal X-irradiation.

In conclusion, we suggest that the long-term changes in cerebellar catecholamine levels induced by X-irradiation at birth are related to the impairment of early postnatal events in the developing cerebellum. Further work is needed to explain the biochemical mechanisms which lead to the production of these alterations.

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