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Neuroanatomical and Functional Alterations Resulting from Early Postnatal Cerebellar Insults in Rodents

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FERGUSON, S. A. *Neuroanatomical and functional alterations resulting from early postnatal cerebellar insults in rodents.* PHARMACOL BIOCHEM BEHAV **55**(4) 663–671, 1996.—This review examines neuroanatomical and functional alterations in rodents resulting from postnatal insults during cerebellar development. Treatments such as irradiation and methylazoxymethanol (MAM) administration produced near birth (< postnatal day 8 for irradiation treatment and < postnatal day 4 for MAM administration) result in more severe cerebellar damage than do similar treatments administered several days after birth. Prominent among the more severe alterations are foliation abnormalities, misalignment of Purkinje cells and continued multiple innervation of climbing fibers; few or none of these occur as a result of later treatments (> postnatal day 8 for irradiation treatment and > postnatal day 4 for MAM treatment). The functional alterations also differ: insults produced near birth result in hypoactivity, ataxia, tremor and accompanying learning deficits, whereas those produced later result in hyperactivity and few learning deficits. This hyperactivity may have relevance to human disorders. Brief discussions of cerebellar and functional alterations (e.g., hyperactivity) resulting from neonatal infection with the Borna disease virus and induction of hypo- and hyperthyroidism during the preweaning period are also presented. **Copyright © 1996 Elsevier Science Inc.**

Cerebellum Development Irradiation Methylazoxymethanol Hypothyroidism Hyperthyroidism Borna disease virus

WITHIN neurobehavioral teratology, rodent studies of disruptions in cerebellar development have focused on those toxicants or insults with the widest clinical relevance (e.g., alcohol exposure). However, there is extensive literature describing neuroanatomical and/or functional effects of cerebellar developmental disruptions that is not often reviewed within the field. These studies described age-dependent principles of cerebellar disruption that might serve to guide future teratological studies. Reviewed here is evidence from acute irradiation and methylazoxymethanol (MAM) treatment studies that suggest developmental stage specificity in severity and type of cerebellar and functional effects. Those insults in the developing rodent produce neuroanatomical and functional consequences that are strikingly different depending on when the insult is produced. Also included in this review are the effects of alterations in thyroid hormone levels during the preweaning period and of neonatal infection with the Borna disease virus. With the exception of focused irradiation, these last three insults also have extracerebellar effects that may contribute to the functional alterations. Not included is a discussion of the genetic mutations in mice (e.g., weaver and staggerer) that can drastically alter cerebellar development because several excellent reviews of this field are available (107,108).

CEREBELLAR DEVELOPMENT IN RODENTS

Cerebellar development in rodents has been thoroughly described (2–5,19,62). The brief review presented here highlights only the neurogenesis and maturation of the different cell populations. Regional differences in developmental rate are well documented in the cerebellum (3,17,19) as are structural/molecular compartmentation differences in the adult cerebellum (63). In general, however, cell types that arise from the ventricular germinal layer originate prenatally, whereas those cell types arising from the external germinal layer are produced postnatally. As development proceeds, the cerebellar cortex acquires three distinctive layers (Fig. 1): (1) the outer molecular layer containing the basket and stellate cells, parallel fibers (axons) of the granule cells and Purkinje cell dendritic trees, (2) the Purkinje cell layer and (3) the inner granule cell body layer (which also contains Golgi cell bodies).

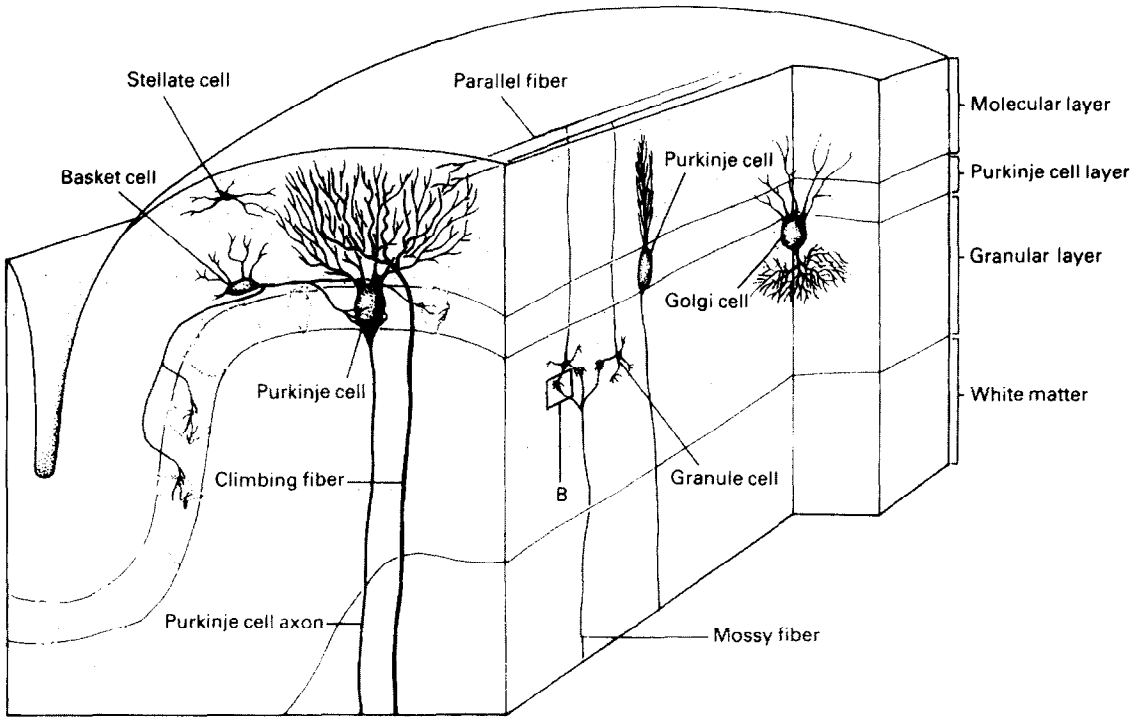

FIG. 1. The layers of an adult cerebellum illustrating the different cell types [reprinted with permission from (53): Ghez, C.; Fahn, S. The cerebellum. In: Kandel, E. R.; Schwartz, J. H., eds. Principles of neural science, 2nd ed. New York: Appleton & Lange (copyright holder); 1985:502–522].

The Purkinje cells and most of the Golgi cells are produced prenatally in the rodent (16,69,96). However, there is substantial maturation of these cell populations during the postnatal period. Purkinje cells form their characteristic monolayer alignment by postnatal day (PND) 4–8 (where day of birth = PND 0†) (3,19,64,97), and their dendritic width and height do not reach adult dimensions until sometime after PND 15 (26,64,97). Synaptogenesis with the parallel fibers of the granule cells is also prolonged, lasting well into the third postnatal week in the rat (3). A temporary innervation of climbing fiber axons from single neurons in the inferior olive onto multiple Purkinje cells occurs, which is maximal at PND 5, and then gradually decreases to the mature innervation of one Purkinje cell by one inferior olive neuron by PND 13–15 (38,94).

The cortical interneurons in the cerebellum include the granule, stellate and basket cell populations, which are produced postnatally in the proliferative zone of the transient external germinal layer. Granule cells greatly outnumber other cerebellar cell types (e.g., ~419:1 ratio of granule to Purkinje cells) (77) and their neurogenesis is especially protracted. Mitotic activity of granule cell precursors begins late (PND 2–4) (4,92) and lasts well into the third postnatal week (4,96), with peak levels between PND 8–15 (4,50). Further maturation includes bifurcation of the granule cell axon, which extends longitudinally, becoming the parallel fibers (2,70). Granule cell bodies migrate inward, guided by Bergmann glial fibers [(105); but see (59) for novel forms of cerebellar granule cell migration] to their final positions in the internal granule cell layer. This migration begins by PND 7 (59,76). Neurogenesis of basket cells occurs mainly on PND 6–7, whereas stellate cells originate later on PND 8–11 (2,16). These cells remain in the molecular layer, developing synapses onto Purkinje cells.

Irradiation

Mitotic cells in the external germinal layer of the cerebellum are very sensitive to ionizing radiation (14,60,71), which causes cell death via increased apoptosis (45,60,119). At least 100 R is necessary to induce cerebellar damage (68), and even a single dose can cause permanent cerebellar alterations (43,49), despite the regenerative capabilities of the external germinal layer (15). Cerebellar-focused irradiation represents one class of insults in which there is a temporal division in the type of neuroanatomical and functional effects.

Neuroanatomical and Functional Alterations after Early Neonatal Irradiation

Rats irradiated during the first postnatal week (before PND 8) show multiple cerebellar abnormalities. Depending on the number of days and intensity of irradiation, these can include decreased size of molecular and granule cell layers (8,12,30,42), decreased numbers of granule and basket cells (8,12,30, 49,93,119), ectopic granule cells in the molecular layer (6, 8,30,93), misorientation of parallel fibers (7,8), severe foliation/lobular malformations (8,12,57,93) and decreased overall size, area or weight of the cerebellum (8,12,42,57), which is most pronounced in the cortical region (43). Although the overall number of Purkinje cells is not affected (12), they do not align in a monolayer (8,12,42,57,93), and their somata can be ectopically located in the granule cell layer or the white

†For the sake of consistency, references cited have been standardized so that day of birth = PND 0.

matter (12,30). In addition, their dendritic trees exhibit abnormal arborization and orientation (6,8,15,30,42,57). Furthermore, the normally transient hyperinnervation by climbing fibers onto single Purkinje cells remains permanent (41,93).

Many of the functional alterations exhibited after irradiation during the first postnatal week are those classically ascribed to cerebellar damage. For example, tremor and ataxia are common (42,57,93,115,117). In addition, there is strong evidence for irradiation-induced hypoactivity. Open field sessions (1–3 min each) have indicated decreased activity and rearing frequencies from preweaning through PND 70 in male rats irradiated during the first postnatal week (13,102,115). Later open field activity (PND 180–184 and 2–2.5 years of age) was comparable to control levels; however, rearing frequencies remained decreased (102,117). Running wheel assessments indicated a similar hypoactivity (116) that dissipated somewhat with age (102). Alterations in strength did not appear to cause or interact with the hypoactivity because weight-pulling ability (up to 300 g) was normal (116).

Development of vertical rope climbing abilities was impaired in young rats and remained poor in adult rats irradiated during the first week postpartum (13,116). Treadmill performance of PND 30 rats was unimpaired; however, maximum jump distance was decreased in adults (31). Rotorod performance, especially at higher speeds concurrent with a smooth surface, and vertical rod climbing were also impaired in adult rats irradiated during the first week postpartum (31,102). Contrary to the performance impairments exhibited in these assessments, swimming ability is normal in male rats irradiated during the first postnatal week (31,102). Irradiation during the first postnatal week also produced cognitive deficits. In double alternation swim mazes, male rats exhibited increased errors and number of trials to criterion (102).

Irradiation schedules that begin during the first postnatal week and extend through PND 15 or 16 also produce hypoactivity, ataxia and tremor and impaired rotorod performance (25,31,56,102). Those effects appear to result from damage produced by the irradiation treatment occurring during the first week because irradiation that begins on PND 8 (and ends on PND 15–16) produces very different effects. In addition, rats irradiated between PND 2–15 and 4–15 show learning deficits in the form of poor acquisition and reversal of position discrimination tasks, impaired spontaneous alteration and poor performance on complex swim mazes, although swimming ability is normal (18,102).

Neuroanatomical and Functional Alterations after Later Neonatal Irradiation

If irradiation is delayed until after the first postnatal week (e.g., PND 9–16, PND 13–16), the characteristic monolayer arrangement of Purkinje cells remains intact (9,10). Overall numbers of basket and Purkinje cells are normal (9,102), and there are only mild abnormalities in Purkinje cell orientation (9). Irradiation schedules beginning after the first postnatal week produce decreased size of molecular and granule cell layers (10,102), with few or no effects on cerebellar foliation (9,10), although overall size can be moderately reduced (9,10,102) (Fig. 2A,B). Irradiation during the second postnatal week, however, severely depletes the granule cell population (up to 78%) (9,102). The continued hyperinnervation by climbing fibers, although still present, is substantially less in rats irradiated during the second postnatal week (41).

Irradiation that begins after the first postnatal week (e.g., PND 8–15, PND 12–15) results in a syndrome of effects that is distinct from that produced by early irradiation. Strikingly different is the hyperactivity produced by these later irradiation paradigms. This effect was described in an elegant series of seven experiments by Pellegrino and Altman (102). Specifically, male rats irradiated later after birth (PND 8–15 or 12–15) were hyperactive and exhibited increased rearing frequencies during the preweaning period (PND 19–21) in 5-min open field sessions. This hyperactivity was still evident at PND 70–72, but activity levels were comparable to control at PND 180–182. Habituation of locomotion in the open field was normal at all ages. Similar patterns were evident at PND 60–90 in running wheel assessments in which rats irradiated at PND 8–15 or PND 12–15 were significantly more active than were controls. Similar to the results from open field assessments, running wheel activity was indistinguishable from control activity at later ages (PND 180–210). Rotorod performance at PND 70 and swimming ability at PND 60 were normal.

Irradiation after the first postnatal week generally spares learning performance. For example, male rats irradiated on PND 8–15 performed normally in single and double alternation swim mazes at PND 60; however, rats irradiated on PND 12–15 were impaired on the double, but not single, alternation swim maze (102). Spontaneous alternation in a T-maze at PND 60–70 was unaffected by irradiation after the first postnatal week (102).

MAM Treatment

MAM is a potent antimitotic compound found in the seeds of the cycad plant (110). Its antimitotic effect lasts only 2–24 h after administration (72); thus, it is typically used to produce hypoplasia in specific neural regions without significantly affecting others. It appears selectively toxic to neuronal cells and exerts its most potent effects on those cells undergoing their final mitosis (33). Unlike irradiation, MAM treatment eliminates cerebellar granule cells via a mechanism other than apoptosis (119). However, both MAM treatment and irradiation exhibit a similar temporal division in type of neuroanatomical and functional effects.

Neuroanatomical and Functional Alterations after Early MAM Treatment

A single MAM injection on the day of birth or a series of injections beginning on that day (but ending on or before PND 4) produces "granuloprival cerebellar hypoplasia" (74,114). Granuloprival cerebellar hypoplasia is characterized by a severe decimation of the granule cell population, reflected in decreased size of the granule cell layer and cell numbers and fewer parallel fibers (35,61,66,91,109,119). In addition, cerebellar cytoarchitecture is disrupted. Although there is little change in the number of Purkinje cells, they fail to orient in a monolayer and are found scattered throughout the molecular and granule cell layers (30,35,61,88). These cells are often misoriented or inverted, and their dendrites grow into the white matter, with numerous unattached postsynaptic thickenings and abnormal spine formations (29,35,40,73,88,120). The multiple innervation by climbing fibers remains present at adulthood (30). Abnormal foliation and alterations in Bergmann glia are common (24,34,35,61,89,103,119).

Several studies have reported ataxia and/or tremors following MAM-induced granuloprival hypoplasia (34,55,58,66,67); quantitative behavioral measures, however, were assessed in two studies. Rats injected on the day of birth were hypoactive in 30-min open field sessions on PND 11–15 but later exhibited normal levels of activity on PND 17–28 (75). Injection of MAM on the first 4 postnatal days resulted in extreme hypoactivity in

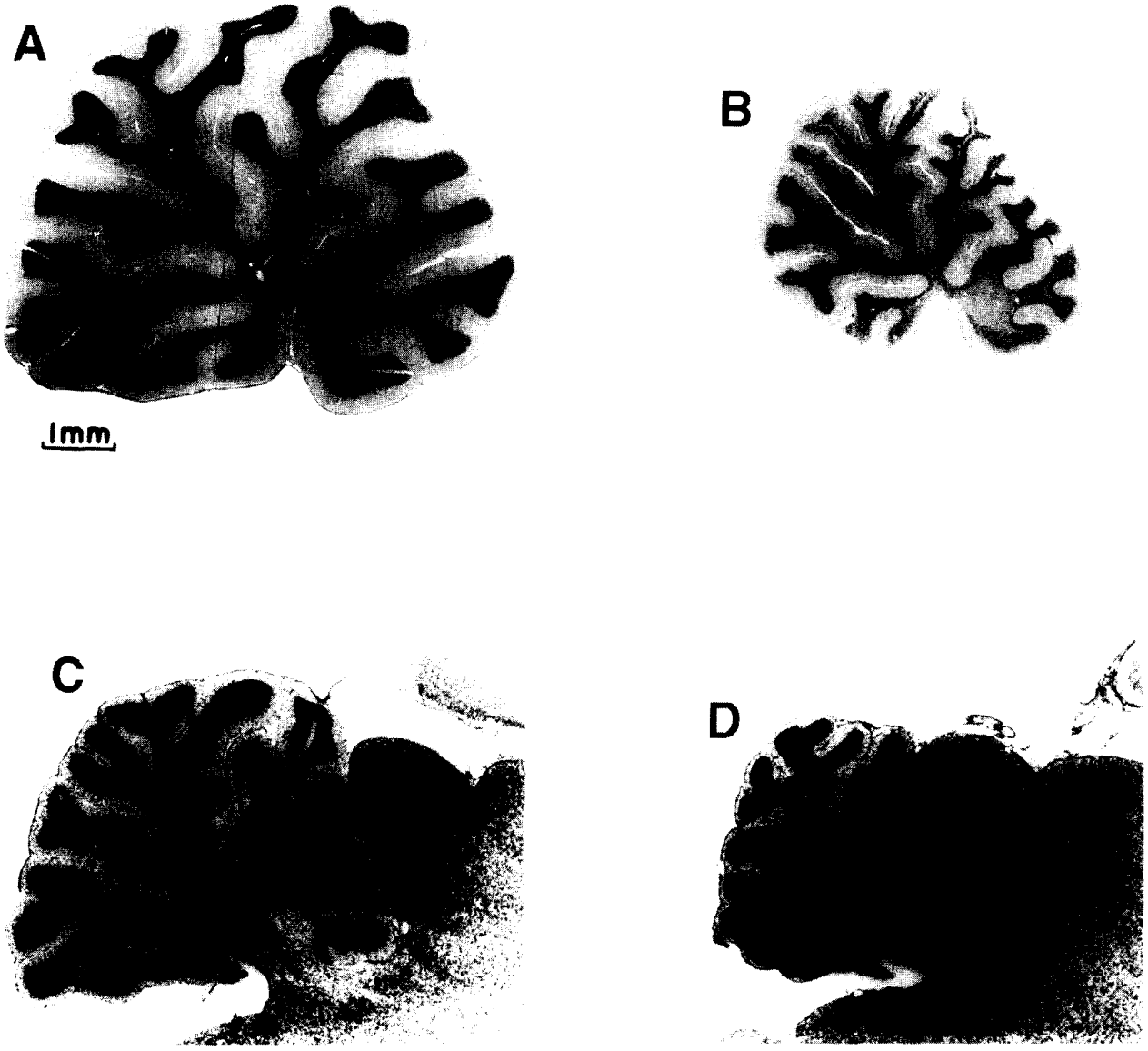

FIG. 2. Midsagittal cerebellar section from a PND 30 control rat (A) and a PND 30 rat irradiated on PND 8–15 (B) [reprinted with permission from (11)]. Parasagittal cerebellar section from a PND 33 control rat (C) and a PND 33 rat treated with MAM on PND 4 and PND 7 (D) [reprinted with permission from (47)]. Note similarities in hypoplasia.

10-min open field assessments on PND 44–46 (78). In both studies, MAM-treated rats displayed approximately 4-day delays in fur growth. Development of the righting reflex and pigmentation were also delayed (78). Rotorod performance at slower speeds (8 rpm) was not impaired; however, at 22 rpm performance of rats treated with MAM on the day of birth was only one-sixth that of controls (75). Because the animals probably had retinal dysplasia as a result of MAM treatment (80), it is unknown whether visual impairments may have been interacted with the results of behavioral assessments.

Neuroanatomical and Functional Alterations after Later MAM Treatment

A more moderate lesion ("hypogranular" cerebellum) is produced by MAM treatment, which begins after PND 4 (52,111). This lesion also results in granule cell depletion with an accompanying decrease in cerebellar weight, but the effects are relatively less severe (Fig. 2C,D). These effects are restricted to the cerebellum (47,103), which has normal foliation (24,51,52,65), relatively minor alterations in Purkinje cell development (65,103) and normal cytoarchitecture (39). The molecular and granule cell layers are reduced in thickness and area (23,47), and there may be ectopic granule cells in the molecular layer (47). There are few or no retinal alterations after this later-treatment paradigm (80).

Two studies have examined functional alterations after MAM-induced hypogranular cerebellum (44,47). Rats treated with MAM on PND 4–7 were mildly hyperactive in 9-min open field assessments on PND 33–36, and this hyperactivity was particularly pronounced in male rats (44). In that study, residential running wheel assessments beginning at PND 50

indicated mild hyperactivity in male, but not female, rats. There were no deficits apparent in adult performance of a 24-arm complex maze or in operant assessments of progressive ratio or temporal response differentiation (44). Similarly, rats treated on PND 4 and 7 with MAM exhibited normal performance of a T-maze delayed alternation on PND 26–27 (47). In that study, conditioned suppression of drinking behavior was normal in PND 26 rats, suggesting unimpaired learning of a conditioned stimulus-unconditioned stimulus association (47). In contrast, associative eyeblink conditioning (using the same conditioned stimulus as that used in conditioned suppression of drinking) was severely impaired in rats with hypogranular cerebellum. This effect was present across different ages (PND 17–32) in which MAM-treated rats did not approach control performance even after 600 conditioning trials (47). The unconditioned eyeblink response, however, was normal.

Hypo- and Hyperthyroidism

Alterations of thyroid hormone levels during the postnatal period can have severe consequences for cerebellar development. Many studies typically treat rats from birth to weaning, and some then allow for a rehabilitation or recovery period.‡

In general, hypothyroidism retards development, whereas hyperthyroidism accelerates it. For example, hypothyroidism slows the migration rate of granule cells from the external germinal layer to the internal granule cell layer and delays the disappearance of the external germinal layer, whereas hyperthyroidism accelerates both these processes (81,83,84). Hypo- or hyperthyroidism results in reduced whole brain and cerebellar weights (measured concurrently with the hypo- or hyperthyroidism) (54,85,98) and a reduced molecular layer (100,106). Parallel fiber development is also disrupted. During hyperthyroidism, the parallel fibers are longer, with increased synaptic capacity; during hypothyroidism, they are shorter and have decreased synaptic capacity (82). Those effects may be permanent because rehabilitated hypothyroid rats have only 60% of the cerebellar synapses relative to controls (106). Hypothyroidism causes an increase in the overall number of granule cells and astrocytes, whereas hyperthyroidism results in fewer granule and basket cells; neither treatment, however, seems to alter the number of Purkinje cells (98,99). The regression of multiple innervation of Purkinje cells by climbing fibers is delayed about 2 days by hypothyroidism (38). Cerebellar foliation is altered in hypothyroid rats as shown by the increased numbers of fissures (84). Overall cerebellar cortical area is reduced, and there is a decreased number of fissures in hyperthyroidism (84).

Behavioral effects resulting from hypothyroidism have been studied more extensively than those resulting from hyperthyroidism, perhaps due to the clinical relevance of iodine deficiency and cretinism (27,90). In both cases, however, altering thyroid hormone levels during the preweaning period followed by recovery results in hyperactivity. For example, hyperthyroidism induced on PND 1–30 or 1–35 resulted in increased locomotor activity and tremors in rats, although it was not clear whether the animals were assessed concurrently or during a recovery period (85,104). Male rats made hypothyroid during PND 0–19 exhibited increased activity and little or no habituation across sessions in an open field at PND 42 (1). Increased activity in an open field or an exploration box on the second test session indicated a similar lack of habituation in adult rats of both sexes that were hypothyroid PND 0–30 (106). Hole board assessments indicated hyperactivity and a lack of habituation in rearing and head-dip activity in PND 42–50 male and female rats made hypothyroid on PND 0–24 (112,113). The habituation deficit appeared to normalize with increasing age; however, increased locomotor activity in a hole board apparatus was still apparent in PND 90–100 rehabilitated male rats (113). Elevated running wheel activity concurrent with normal circadian rhythms in activity were apparent in rehabilitated male rats made hypothyroid on PND 0–24 and tested at PND 42–50 or PND 90–100 (113).

Rehabilitated male rats (hypothyroidism on PND 0–19) show learning deficits reflected in increased latency to reach the goal and increased errors in a Biel water maze (1). Those deficits were unrelated to swimming difficulties because there were no differences in swimming time on a straight course. In that study, the rehabilitated rats also exhibited extremely poor performance on a radial arm maze. During the training period for that task, many of the previously hypothyroid rats did not consume the food pellets placed at the end of maze arms. After training, rehabilitated rats made many working errors in the radial maze, revisiting previously visited arms (no reference memory measure was available because all arms were baited) (1). Similarly, hyperthyroidism during the preweaning period resulted in increased working memory errors in adult male rats in a radial-arm maze; reference memory, however, was unimpaired (101). Performance of a differential reinforcement of low rate (DRL) operant schedule was not statistically different between previously hypothyroid (induced on PND 0–30) adult rats of both sexes and controls; however, rehabilitated rats improved somewhat slower across sessions (106). The latter effect might indicate a mild failure to withhold responses that would be consistent with a hyperactivity syndrome. Passive avoidance learning appears unimpaired in previously hypothyroid male and female rats (induced on PND 0–24) (112,113). In that study, active avoidance learning in which the conditioned response was to jump onto a platform was also unimpaired in rehabilitated female rats, although they performed the conditioned response more frequently during the intertrial intervals, possibly indicating a form of hyperactivity (112). However, active avoidance learning was impaired in previously hypothyroid (induced on PND 0–30) rats of both sexes, such that the rehabilitated rats learned the avoidance response (a lever press) much more slowly than did controls (106). In the same study, learning to escape shock in a T-maze was also impaired. Because the escape paradigm was part of a reversal series in which the first shock presented required escape in the nonpreferred direction, it was not clear if the deficit reflected impaired spatial or escape learning.

Borna Disease Viral Infection

Viral infection can cause alterations in neural development. Of relevance to humans, the presence of antibodies to Borna disease virus has been associated with depression and schizophrenia (48,118). Rats infected with Borna disease virus as adults typically develop the neurologic signs of the disease such as encephalitis; however, if infection occurs within 48 h of birth, no neurologic signs of Borna disease become apparent (21,22,32). Cerebellar alterations in the "persistant, tolerant

‡Not included in this review are studies in which the thyroid hormone alteration treatment began prenatally (e.g., Goldey et al., 1995; Albee et al., 1989).

infection-newborn rat" include hypoplasia, decreased foliation, decreased size of the internal granule cell layer and premature loss of the external granular layer (21,32). Coordination and motor skills appeared unimpaired (32). However, PND 32 and PND 115 infected male and female rats were hyperactive in a 26-h open field session, with the greatest increase in activity occurring during the first 2-h period (22). Salt taste preferences were altered in PND 60 infected male and female rats; the rats exhibited an increased preference for a saline solution when it was paired with either water, saccharin or quinine solutions (22).

SUMMARY

Results from studies in which cerebellar development was disrupted via irradiation or MAM treatment reveal a temporal division in the severity of neuroanatomical alterations and type of functional alterations. Insults produced near birth (during the first week postpartum for irradiation and before PND 4 for MAM treatment) result in the misalignment of Purkinje cells and continued multiple innervation by climbing fibers and by those behaviors typically described as cerebellar in nature such as ataxia, tremors, hypoactivity and learning deficits. Later developmental insults result in milder cerebellar effects (i.e., normal foliation, minor alterations in Purkinje cell development). However, these later insults produce a syndrome of hyperactivity with few learning deficits. The hyperactivity resulting from later irradiation or MAM treatment is similar to that produced by neonatal infection with the Borna disease virus or alterations of thyroid hormone levels during the preweaning period. However, the learning deficits resulting from early irradiation or MAM treatment may be similar to those produced by alterations of thyroid hormone levels during the preweaning period. Thus, alterations of thyroid hormone levels and, to a lesser extent, infection with the Borna disease virus, produce behavioral alterations that cross the temporal division as described for irradiation and MAM treatment.

This review should serve to emphasize the exquisite sensitivity of the developing cerebellum. This fact alone should direct increased attention to this structure particularly because so much of this development occurs postnatally in the rodent. Insults during this period are easily produced, and further research might clarify the neuroanatomical and/or neurochemical substrates underlying the striking differences in behavioral alterations.

The studies reviewed here are particularly relevant in light of the accumulating evidence indicating cerebellar involvement in higher cognitive functioning (28,46,86), emotion [reviewed in (79)] and language [reviewed by (87)]. In addition, there is intriguing evidence suggesting that cerebellar alterations might play a role in such human disorders as autism (20,36,37) and schizophrenia [reviewed in (95)]. Certainly, the cerebellum is more than simply a structure involved in motor sequences and coordination.

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REFERENCES

1. Akaike, M.; Kato, N.; Ohno, H.; Kobayashi, T. Hyperactivity and spatial maze learning impairment of adult rats with temporary neonatal hypothyroidism. Neurotoxicol. Teratol. 13:317–322; 1991.
2. Altman, J. Postnatal development of the cerebellar cortex in the rat. I. The external granular layer and the transitional molecular layer. J. Comp. Neurol. 145:353–398; 1972.
3. Altman, J. Postnatal development of the cerebellar cortex in the rat. II. Phases in the maturation of Purkinje cells and of the molecular layer. J. Comp. Neurol. 145:399–464; 1972.
4. Altman, J. Postnatal development of the cerebellar cortex in the rat. I. Maturation of the components of the granular layer. J. Comp. Neurol. 145:465–514; 1972.
5. Altman, J. Postnatal development of the cerebellar cortex in the rat. IV. Spatial organization of bipolar cells, parallel fibers, and glial palisades. J. Comp. Neurol. 163:427–448; 1975.
6. Altman, J. Experimental reorganization of the cerebellar cortex. I. Regeneration of the external germinal layer and granule cell ectopia. J. Comp. Neurol. 149:153–180; 1973.
7. Altman, J. Experimental reorganization of the cerebellar cortex. IV. Parallel fiber reorientation following regeneration of the external germinal layer. J. Comp. Neurol. 149:181–192; 1973.
8. Altman, J. 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; 1976.
9. Altman, J. 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; 1976.
10. Altman, J. 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; 1976.
11. Altman, J. Morphological and behavioral markers of environmentally induced retardation of brain development: An animal model. Environ. Health Perspect. 74:153–168; 1987.
12. Altman, J.; Anderson, W. J. Irradiation of the cerebellum of infant rats with low-level x-ray: Histological and cytological effects during infancy and adulthood. Exp. Neurol. 30:492–509; 1971.
13. Altman, J.; Anderson, W. J.; Strop, M. Retardation of cerebellar and motor development by focal x-irradiation during infancy. Physiol. Behav. 7:143–150; 1971.
14. Altman, J.; Anderson, W. J.; Wright, K. A. Differential radiosensitivity of stationary and migratory primitive cells in the brains of infant rats. Exp. Neurol. 22:52–74; 1968.
15. Altman, J.; Anderson, W. J.; Wright, K. A. Reconstitution of the external granular layer of the cerebellar cortex in infant rats after low-level x-irradiation. Anat. Rec. 163:453–472; 1969.
16. Altman, J.; Bayer, S. A. 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. 179:23–48; 1978.
17. Altman, J.; Bayer, S. A. Embryonic development of the rat cerebellum. I. Regional differences in time of origin, migration, and settling of Purkinje cells. J. Comp. Neurol. 231:42–65; 1985.
18. Altman, J.; Brunner, R. L.; Bulut, F. G.; Sudarshan, K. The development of behavior in normal and brain-damaged infant rats, studied with homing (nest-seeking) as motivation. In: Vernadakis, A.; Weiner, N., eds. Advances in behavioral biology, vol. 8. Drugs and the developing brain. New York: Plenum Press; 1974:321–348.
19. Altman, J.; Winfree, J. Postnatal development of the cerebellar cortex in the rat. V. Spatial organization of Purkinje cell perikarya. J. Comp. Neurol. 171:1–16; 1977.

20. Bauman, M. L. Microscopic neuroanatomic abnormalities in autism. Pediatrics. 87 (Suppl.):791–796; 1991.
21. Bautista, J. R.; Rubin, S. A.; Moran, T. H.; Schwartz, G. J.; Carbone, K. M. Developmental injury to the cerebellum following perinatal Borna disease virus infection. Dev. Brain Res. 90:45–53; 1995.
22. Bautista, J. R.; Schwartz, G. J.; De La Torre, J. C.; Moran, T. H.; Carbone, K. M. Early and persistent abnormalities in rats with neonatally acquired Borna disease virus infection. Brain Res. Bull. 34:31–40; 1994.
23. Becker, T.; Gombos, G.; de Barry, J. Differential ontogenic pattern of metabotropic [^{3}H]-L-glutamate receptors in normal and granule cell-deficient mouse cerebellum. Exp. Brain Res. 107:361–366; 1996.
24. Bejar, A.; Roujansky, A.; de Barry, J.; Gombos, G. Different effect of methylazoxymethanol on mouse cerebellar development depending on the age of injection. Exp. Brain Res. 57:279–285; 1985.
25. Benoit, P.; Delhaye-Bouchard, N.; Changeux, J.; Mariani, J. Stability of multiple innervation of Purkinje cells by climbing fibers in the agranular cerebellum of old rats x-irradiated at birth. Dev. Brain Res. 14:310–313; 1984.
26. Berry, M.; Bradley, P. The growth of the dendritic trees of Purkinje cells in the cerebellum of the rat. Brain Res. 112:1–35; 1976.
27. Boyages, S. C. Clinical review 49: Iodine deficiency disorders. J. Clin. Endocrinol. Metab. 77:587–591; 1993.
28. Bracke-Tolkmitt, R.; Linden, A.; Canavan, A. G. M.; Rockstroh, B.; Scholz, E.; Wessel, K.; Diener, H.-C. The cerebellum contributes to mental skills. Behav. Neurosci. 103:442–446; 1989.
29. Bradley, P.; Berry, M. Quantitative effects of methylazoxymethanol acetate on Purkinje cell dendritic growth. Brain Res. 143:499–511; 1978.
30. Bravin, M.; Rossi, F.; Strata, P. Different climbing fibres innervate separate dendritic regions of the same Purkinje cell in hypogranular cerebellum. J. Comp. Neurol. 357:395–407; 1995.
31. Brunner, R. L.; Altman, J. Locomotor deficits in adult rats with moderate to massive retardation of cerebellar development during infancy. Behav. Biol. 9:169–188; 1973.
32. Carbone, K. M.; Park, S. W.; Rubin, S. A.; Waltrip, R. W., II; Vogelsang, G. B. Borna Disease: Association with a maturation defect in the cellular immune response. J. Virol. 65:6154–6164; 1991.
33. Cattaneo, E.; Reinach, B.; Caputi, A.; Cattabeni, F.; Di Luca, M. Selective in vitro blockage of neuroepithelial cells proliferation by methylazoxymethanol, a molecule capable of inducing long lasting functional impairments. J. Neurosci. Res. 41:640–647; 1995.
34. Chen, S.; Hillman, D. E. Selective ablation of neurons by methylazoxymethanol during pre- and postnatal brain development. Exp. Neurol. 94:103–119; 1986.
35. Chen, S.; Hillman, D. E. Regulation of granule cell number by a predetermined number of Purkinje cells in development. Dev. Brain Res. 45:137–147; 1989.
36. Courchesne, E. Neuroanatomical systems involved in infantile autism: The implications of cerebellar abnormalities. In: Dawson, G., ed. Autism: New perspectives on diagnosis, nature and treatment. New York: Guilford Press; 1989:119–143.
37. Courchesne, E.; Saitoh, O.; Yeung-Courchesne, R., Press, G. A.; Lincoln, A. J.; Haas, R. H.; Schreibman, L. Abnormality of cerebellar vermian lobules VI and VII in patients with infantile autism: Identification of hypoplastic and hyperplastic subgroups by MR imaging. Am. J. Roentgenol. 162:123–130; 1994.
38. Crepel, F.; Delhaye-Bouchard, N.; Dupont, J. L. Fate of the multiple innervation of cerebellar Purkinje cells by climbing fibers in immature control, x-irradiated and hypothyroid rats. Dev. Brain Res. 1:59–71; 1981.
39. de Barry, J.; Gombos, G. Immunohistochemistry with anti- calbindin and anti-neurofilament antibodies in the cerebellum of methylazoxymethanol-treated mice. J. Neurosci. Res. 23:330–336; 1989.
40. de Barry, J.; Gombos, G.; Klupp, T.; Hamori, J. Alteration of mouse cerebellar circuits following methylazoxymethanol treatment during development: Immunohistochemistry of GABAergic elements and electron microscopic study. J. Comp. Neurol. 261:253–265; 1987.
41. Delhaye-Bouchaud, N.; Mory, G.; Crepel, F. Differential role of granule cells in the specification of synapses between climbing fibers and cerebellar Purkinje cells in the rat. Neurosci. Lett. 9:51–58; 1978.
42. Dopico, A. M.; Rios, H.; Mayo, J.; Zieher, L. M. Increased activity of tyrosine hydroxylase in the cerebellum of the x-irradiated dystonic rat. Mol. Chem. Neuropathol. 13:129–143; 1990.
43. Dopico, A. M.; Zieher, L. M. Neurochemical characterization of the alterations in the noradrenergic afferents to the cerebellum of adults rats exposed to x-irradiation at birth. J. Neurochem. 61:481–489; 1993.
44. Ferguson, S. A.; Paule, M. G.; Holson, R. R. Functional effects of methylazoxymethanol-induced cerebellar hypoplasia in rat. Neurotoxicol. Teratol., in press.
45. Ferrer, I.; Serrano, T.; Rivera, R.; Olive, M.; Zujar, M. J.; Graus, F. Radiosensitive populations and recovery in x-ray-induced apoptosis in the developing cerebellum. Acta Neuropathol. 86:491–500; 1993.
46. Fiez, J. A. Cerebellar contributions to cognition. Neuron 16:13–15; 1996.
47. Freeman, J. H.; Barone, S., Jr.; Stanton, M. E. Disruption of cerebellar maturation by an antimitotic agent impairs the ontogeny of eyeblink conditioning in rats. J. Neurosci. 15:7301–7314; 1995.
48. Fu, Z. F.; Amsterdam, J. D.; Kao, M.; Shankar, V.; Koprowski, H.; Dietzschold, B. Detection of Borna disease virus-reactive antibodies from patients with affective disorders by Western immunoblot technique. J. Affect. Disord. 27:61–68; 1993.
49. Fuhrman, Y.; Thomson, M. A.; Piat, G.; Mariani, J.; Delhaye-Bouchard, N. Enlargement of olivo-cerebellar microzones in the agranular cerebellum of adult rats. Brain Res. 638:277–284; 1994.
50. Fujita, S.; Shimada, M.; Nakamura, T. H^3-thymidine autoradiographic studies on the cell proliferation and differentiation in the external and the internal granular layers of the mouse cerebellum. J. Comp. Neurol. 128:191–208; 1966.
51. Garcia-Ladona, F. J.; de Barry, J.; Girard, C.; Gombos, G. Ectopic granule cell layer in mouse cerebellum after methyl-azoxymethanol (MAM) treatment. Exp. Brain Res. 86:90–96; 1991.
52. Garcia-Ladona, F. J.; Palacios, J. M.; Girard, C.; de Barry, J.; Gombos, G. Autoradiographic localization of [^{3}H]-L- glutamate binding sites in a model of cerebellar granule cell ectopia generated by methylazoxymethanol treatment. J. Chem. Neuroanat. 6:323–329; 1993.
53. Ghez, C.; Fahn, S. The cerebellum. In: Kandel, E. R.; Schwartz, J. H., eds. Principles of neural science, 2nd ed. New York: Elsevier; 1985:502–522.
54. Gourdon, J.; Clos, J.; Coste, C.; Dainat, J.; Legrand, J. Comparative effects of hypothyroidism, hyperthyroidism and undernutrition on the protein and nucleic acid contents of the cerebellum in the young rat. J. Neurochem. 21:861–871; 1973.
55. Grondin, G.; Sharkey, T.; Jones, M.; Sculthorpe, A.; Taylor, W. Postnatal cerebellar hypoplasia and dysfunction following methylazoxymethanol acetate treatment. Proc. Soc. Exp. Biol. Med. 148:156–159; 1975.
56. Gruner, J. A.; Altman, J.; Spivack, N. Effects of arrested cerebellar development on locomoion in the rat. Exp. Brain Res. 40:361–373; 1980.
57. Guelman, L. R.; Zieher, L. M.; Rios, H.; Mayo, J.; Dopico, A. M. Motor abnormalities and changes in the noradrenaline content and the cytoarchitecture of developing cerebellum following x-irradiation at birth. Mol. Chem. Neuropathol. 20: 45–57; 1993.
58. Haddad, R. K.; Rabe, A.; Dumas, R. Comparison of effects of methylazoxymethanol acetate on brain development in different species. Fed. Proc. 31:1520–1523; 1972.
59. Hager, G.; Dodt, H.-U.; Zieglgansberger, W.; Liesi, P. Novel forms of neuronal migration in the rat cerebellum. J. Neurosci. Res. 40:207–219; 1995.

60. Harmon, B. V.; Allan, D. J. X-ray-induced cell death by apoptosis in the immature rat cerebellum. Scan. Microsc. 2:561–568; 1988.
61. Hartkop, T. H.; Jones, M. Z. Methylazoxymethanol-induced aberrant Purkinje cell dendritic development. J. Neuropathol. Exp. Neurol. 36:519–532; 1977.
62. Hatten, M. E.; Heintz, N. Mechanisms of neural patterning and specification in the developing cerebellum. Annu. Rev. Neurosci. 18:385–408; 1995.
63. Hawkes, R.; Blyth, S.; Chockkan, V.; Tano, D.; Ji, Z.; Mascher, C. Structural and molecular compartmentation in the cerebellum. Can. J. Neurol. Sci. 20 (Suppl.):S29-S35; 1993.
64. Hendelman, W. J.; Aggerwal, A. S. The Purkinje neuron: I. A Golgi study of its development in the mouse and in culture. J. Comp. Neurol. 193:1063–1079; 1980.
65. Hillman, D. E.; Chen, S.; Ackman, J. Perinatal methylazoxymethanol acetate uncouples coincidence of orientation of cerebellar folia and parallel fibers. Neuroscience 24:99–110; 1988.
66. Hirono, A.; Jones, M. Fine structure of cycasin-induced cerebellar alterations. Fed. Proc. 31:1517–1519; 1972.
67. Hirono, I.; Shibuya, C.; Hayashi, K. Induction of a cerebellar disorder with cycasin in newborn mice and hamsters. Proc. Soc. Exp. Biol. Med. 131:593–599; 1969.
68. Inouye, M.; Kameyama, Y. Long-term neuropathological consequences of low-dose x-irradiation on the developing rat cerebellum. J. Radiat. Res. 27:240–246; 1986.
69. Inouye, M.; Murakami, U. Temporal and spatial patterns of Purkinje cell formation in the mouse cerebellum. J. Comp. Neurol. 194:499–503; 1980.
70. Ito, M. The cerebellum and neural control. New York: Raven Press; 1984.
71. Jensen, K. F.; Altman, J. Radiosensitivity of the granule cell line and other cell types of the immature rat cerebellar cortex. Exp. Neurol. 77:113–128; 1982.
72. Johnston, M. V.; Coyle, J. T. Cytotoxic lesions and the development of transmitter systems. Trends Neurosci. 5:153–156; 1982.
73. Jones, M. Z.; Gardner, E. Pathogenesis of methylazoxymethanol-induced lesions in the postnatal mouse cerebellum. J. Neuropathol. Exp. Neurol. 35:413–444; 1976.
74. Jones, M.; Yang, M.; Mickelsen, O. Effects of methylazoxymethanol glucoside and methylazoxymethanol acetate on the cerebellum of the postnatal Swiss albino mouse. Fed. Proc. 31:1508–1511; 1972.
75. Kabat, K.; Buterbaugh, G. G.; Eccles, C. U. Methylazoxymethanol as a developmental model of neurotoxicity. Neurobehav. Toxicol. Teratol. 7:519–525; 1985.
76. Komuro, H.; Rakic, P. Dynamics of granule cell migration: A confocal microscopic study in acute cerebellar slice preparations. J. Neurosci. 15:1110–1120; 1995.
77. Korbo, L.; Andersen, B. B.; Ladefoged, O.; Moller, A. Total numbers of various cell types in rat cerebellar cortex estimated using an unbiased stereological methods. Brain Res. 609:262–268; 1993.
78. Lai, H.; Quock, R. M.; Makous, W.; Horita, A.; Jen, L. S. Methylazoxymethanol acetate: Effect of postnatal injection on brain amines and behavior. Pharmacol. Biochem. Behav. 8:251–257; 1978.
79. Lalonde, R.; Botez, M. I. The cerebellum and learning processes in animals. Brain Res. Rev. 15:325–332; 1990.
80. Latker, C. H.; Eiden, L. E.; Zatz, M. The effect of methylazoxymethanol acetate (MAM) on the developing rat retina. Exp. Eye Res. 35:351–361; 1982.
81. Lauder, J. M. The effects of early hypo- and hyperthyroidism on the development of rat cerebellar cortex. I. Kinetics of cell proliferation in the external granular layer. Brain Res. 126: 31–51; 1977.
82. Lauder, J. M. Effects of early hypo- and hyperthyroidism on development of rat cerebellar cortex. IV. The parallel fibers. Brain Res. 142:25–39; 1978.
83. Lauder, J. M. Granule cell migration in developing rat cerebellum. Dev. Biol. 70:105–115; 1979.
84. Lauder, J. M.; Altman, J.; Krebs, H. Some mechanisms of cerebellar foliation: Effects of early hypo- and hyperthyroidism. Brain Res. 76:33–40; 1974.
85. Legrand, L.; Selme-Matrat, M.; Rabie, A.; Clos, J.; Legrand, C. Thyroid hormone and cell formation in the developing rat cerebellum. Biol. Neonate 29:368–380; 1976.
86. Leiner, H. C.; Leiner, A. L.; Dow, R. S. Does the cerebellum contribute to mental skills? Behav. Neurosci. 100:443–454; 1986.
87. Leiner, H. C.; Leiner, A. L.; Dow, R. S. Cognitive and language functions of the human cerebellum. Trends Neurosci. 16:444–447; 1993.
88. Lovell, K. L.; Jones, M. Z. Partial external germinal layer regeneration in the cerebellum following methylazoxymethanol administration: Effects on Purkinje cell dendritic spines. J. Neuropathol. Exp. Neurol. 39:541–548; 1980.
89. Luthman, J.; Eriksdotter-Nilsson, M.; Jonsson, G. Structural and neurochemical effects in mouse cerebellum following neonatal methylazoxymethanol and 6-hydroxydopamine treatment. Int. J. Dev. Neurosci. 8:107–118; 1990.
90. Maberly, G. F. Iodine deficiency disorders: Contemporary scientific issues. J. Nutr. 124:1473S–1478S; 1994.
91. Makowiec, R. L.; Cha, J. J.; Penney, J. B.; Young, A. B. Cerebellar excitatory amino acid binding sites in normal, granuloprival, and Purkinje cell-deficient mice. Neuroscience 42:671–681; 1991.
92. Mares, V.; Lodin, Z.; Srajer, J. The cellular kinetics of the developing mouse cerebellum. I. The generation cycle, growth fraction and rate of proliferation of the external granular layer. Brain Res. 23:323–342; 1970.
93. Mariani, J.; Benoit, P.; Duc Hoang, M.; Thomson, M.; Delhaye-Bouchaud, N. Extent of multiple innervation of cerebellar Purkinje cells by climbing fibers in adult x-irradiated rats: Comparison of different schedules of irradiation during the first postnatal week. Dev. Brain Res. 57:63–70; 1990.
94. Mariani, J.; Changeux, J. Ontogenesis of olivocerebellar relationships. I. Studies by intracellular recordings of the multiple innervation of Purkinje cells by climbing fibers in the developing rat cerebellum. J. Neurosci. 1:696–702; 1981.
95. Martin, P.; Albers, M. Cerebellum and schizophrenia: A selective review. Schizophr. Bull. 21:241–250; 1995.
96. Miale, I. L.; Sidman, R. L. An autoradiographic analysis of histogenesis in the mouse cerebellum. Exp. Neurol. 4:277–296; 1961.
97. Mjaatvedt, A. E.; Wong-Riley, M. T. T. Relationship between synaptogenesis and cytochrome oxidase activity in Purkinje cells of the developing rat cerebellum. J. Comp. Neurol. 277:155–182; 1988.
98. Nicholson, J. L.; Altman, J. The effects of early hypo- and hyperthyroidism on the development of rat cerebellar cortex. I. Cell proliferation and differentiation. Brain Res. 44:13–23; 1972.
99. Nicholson, J. L.; Altman, J. The effects of early hypo- and hyperthyroidism on the development of rat cerebellar cortex. II. Synaptogenesis in the molecular layer. Brain Res. 44:25–36; 1972.
100. Nicholson, J. L.; Altman, J. Synaptogenesis in the rat cerebellum: Effects of early hypo- and hyperthyroidism. Science 176:530–532; 1972.
101. Pavlides, C.; Westlind-Danielsson, A. I.; Nyborg, H.; McEwen, B. S. Neonatal hyperthyroidism disrupts hippocampal LTP and spatial learning. Exp. Brain Res. 85:559–564; 1991.
102. Pelligrino, L. J.; Altman, J. Effects of differential interference with postnatal cerebellar neurogenesis on motor performance, activity level, and maze learning of rats: A developmental study. J. Comp. Physiol. Psychol. 93:1–33; 1979.
103. Rabie, A.; Selme-Matrat, M.; Clavel, M-C.; Clos, J.; Legrand, J. Effects of methylazoxymethanol given at different stages of postnatal life on development of the rat brain: Comparison with those of thyroid deficiency. J. Neurobiol. 8:337–354; 1977.
104. Rastogi, R. B.; Singhal, R. L. Influence of neonatal and adult hyperthyroidism on behavior and biosynthetic capacity for norepinephrine, dopamine and 5-hydroxytryptamine in rat brain. J. Pharmacol. Exp. Ther. 198:609–618; 1976.
105. Rivas, R. J.; Hatten, M. E. Motility and cytoskeletal organization of migrating cerebellar granule neurons. J. Neurosci. 15:981–989; 1995.
106. Schalock, R. L.; Brown, W. J.; Smith, R. L. Long-term effects

of propylthiouracil-induced neonatal hypothyroidism. Dev. Psychobiol. 12:187–199; 1979.
107. Sidman, R. L. Experimental neurogenetics. In: Kety, S. S.; Rowland, L. P.; Sidman, R. L.; Matthysse, S. W., eds. Genetics of neurological and psychiatric disorders. New York: Raven Press; 1983:19–46.
108. Sidman, R. L. Neuropathology, mutant studies. In: Adelman, G., ed. Encyclopedia of neuroscience. Boston: Birkhauser; 1987:828–832.
109. Slevin, J. T.; Johnston, M. V.; Biziere, K.; Coyle, J. T. Methylazoxymethanol acetate ablation of mouse cerebellar granule cells: Effects on synaptic neurochemistry. Dev. Neurosci. 5:3–12; 1982.
110. Spencer, P. S. Are neurotoxins driving us crazy? Planetary observations on the causes of neurodegenerative diseases of old age. In: Russell, R. W.; Flattau, P. E.; Pope, A. M., eds. Behavioral measures of neurotoxicity. Washington, DC: National Academy Press; 1990:11–36.
111. Sullivan-Jones, P.; Ali, S. F.; Gough, B.; Holson, R. R. Postnatal methylazoxymethanol: Sensitive periods and regional selectivity of effects. Neurotoxicol. Teratol. 16:631–637; 1994.
112. Tamasy, V.; Meisami, E.; Du, J. Z.; Timiras, P. S. Exploratory behavior, learning ability, and thyroid hormonal responses to stress in female rats rehabilitating from postnatal hypothyroidism. Dev. Psychobiol. 19:537–553; 1986.
113. Tamasy, V.; Meisami, E.; Vellerga, A.; Timiras, P. S. Rehabilitation from neonatal hypothyroidism: Spontaneous motor activity, exploratory behavior, avoidance learning and responses of pituitary-thyroid axis to stress in male rats. Psychoneuroendocrinology 11:91–103; 1986.
114. Turgeon, S. M.; Albin, R. L. Pharmacology, distribution, cellular localization, and development of GABAB binding in rodent cerebellum. Neuroscience 55:311–323; 1993.
115. Wallace, R. B.; Altman, J. Behavioral effects of neonatal irradiation of the cerebellum. I. Qualitative observations in infant and adolescent rats. Dev. Psychobiol. 2:257–265; 1970.
116. Wallace, R. B.; Altman, J. Behavioral effects of neonatal irradiation of the cerebellum. II. Quantitative studies in young-adult and adult rats. Dev. Psychobiol. 2:266–272; 1970.
117. Wallace, R. B.; Daniels, C. E.; Altman, J. Behavioral effects of neonatal irradiation of the cerebellum. I. Qualitative observations in aged rats. Dev. Psychobiol. 5:35–41; 1972.
118. Waltrip, R. W., II; Buchanan, R. W.; Summerfelt, A.; Breier, A.; Carpenter, W. T., Jr.; Bryant, N. L.; Rubin, S. A.; Carbone, K. M. Borna disease virus and schizophrenia. Psychiatry Res. 56:33–44; 1995.
119. Wood, K. A.; Youle, R. J. The role of free radicals and p53 in neuron apoptosis in vivo. J. Neurosci. 15:5851–5857; 1995.
120. Woodward, D. J.; Bickett, D.; Chanda, R. Purkinje cell dendritic alterations after transient developmental injury of the external granular layer. Brain Res. 97:195–214; 1975.