Eur J Pediatr (2010) 169:1517–1522
DOI 10.1007/s00431-010-1258-8

ORIGINAL PAPER

Maternal stress induces long-lasting Purkinje cell developmental impairments in mouse offspring

Rodrigo Pascual · Daniela Ebner · Rodrigo Araneda · María José Urqueta · Carlos Bustamante

Received: 10 May 2010 / Accepted: 7 July 2010 / Published online: 23 July 2010

Abstract A number of clinical studies suggest that prenatal stress can be a risk factor in the development of various psychopathologies, including schizophrenia, depression, anxiety, and autism. The cerebellar vermis has been shown to be involved in most of these disorders. In the present study, therefore, we evaluate the effect of maternal stress on long-term alterations in vermal Purkinje cell morphology. Furthermore, to discern whether these structural changes are associated with anxious behavior, the exploratory drive in the elevated plus maze was evaluated. Pregnant CF-1 mice were randomly assigned to control (n=14) or stressed (n=16) groups. Dams of the stressed group were subjected to restraint stress between gestational days 14 and 20, while control pregnant dams remained undisturbed in their home cages. Anxious behavior and Purkinje cell morphology were evaluated in three ontogenetic stages: postweaning, adolescence, and adulthood. Although exploratory behavior in the elevated plus maze was unaffected by prenatal stress, the Purkinje cell morphology showed a transient period of abnormal growth (at postweaning and juvenile stages) followed by dramatic dendritic atrophy in adulthood. In conclusion, prenatal stress induced significant long-lasting bimodal changes in the morphology of vermal Purkinje cells. These structural alterations, however, were not accompanied by anxious behaviors in the elevated plus maze.

Keywords Prenatal stress · Purkinje cell development · Elevated plus maze · Golgi method · Anxious behavior

R. Pascual (✉) · D. Ebner · R. Araneda · M. J. Urqueta · C. Bustamante
Laboratorio de Neurociencias, Escuela de Kinesiología,
Facultad de Ciencias,
Pontificia Universidad Católica de Valparaíso,
Valparaíso, Chile
e-mail: rodrigo.pascual@ucv.cl

Introduction

Several preclinical and clinical studies have provided substantial evidence to support the involvement of the cerebellum in various cognitive and emotional dysfunctions. Of particular interest is the relationship between vermal cerebellar abnormalities and certain psychiatric illnesses, including attention deficit hyperactivity disorder, schizophrenia, bipolar disorder, depression, anxiety, and autism [5, 12, 18, 20, 21, 23, 25, 32, 35, 39]. The relationship between these psychopathologies and cerebellar structures are not surprising when one considers that the medial (vermal) cerebellum directly or indirectly establishes a considerable number of connections with the limbic system (i.e., hypothalamus, amygdala, hippocampus, and prefrontal cortex) [28, 37, 44]. Although the causes of most psychiatric disorders are currently unknown, at least one of the risk factors that appear recurrently in the literature is prenatal stress (PS). It has long been recognized that PS increases the susceptibility to behavioral disorders in adolescence or adulthood [43, 44] as well as a long-term reduction in cerebral and cerebellar gray matter volumes [8].

Consistent with the clinical and pathological findings mentioned above, studies performed in animal models have shown that cerebellar cortical neurons are vulnerable to early adverse experiences. For example, it has been shown that vermal Purkinje cells of rats submitted to social isolation stress during the early postweaning period display significant dendritic atrophy, reduction in dendritic spine density and reduced expression of the neuroprotective protein calbindin-D28k [29, 30, 33]. Currently, only two studies have examined the impact of maternal adverse experiences on cerebellar cytoarchitecture development in offspring. These studies show that PS can cause granular cell soma atrophy and decreased synaptic density [40, 41].

Granular cells establish a large number of synaptic connections with the Purkinje cell dendritic tree, eliciting powerful neurotrophic effects [4, 7, 22]. In light of these findings, it seems likely that PS alters the development of these inhibitory projection neurons. The morphological changes occurring in cerebellar Purkinje cells during brain development are of great functional importance: they integrate the two main afferents pathways (i.e., mossy-parallel fibers and climbing fibers) that impinge on Purkinje cell dendritic tree from widespread brain regions and form the sole output from the cerebellar cortex to deep cerebellar nuclei. Thus, cerebellar Purkinje cells act as major integration-processing regulatory units that "supervise" extensive neuronal brain networks [37].

The purpose of the present study was to evaluate whether PS alters the maturation of Purkinje cells and to determine whether these morphological alterations are transient or permanent. For this purpose, we evaluated the impact of PS on dendritic development of vermal Purkinje cells in mice at three ontogenetic stages: postweaning (P22), adolescence (P52), and adulthood (P82). In addition, because the cerebellar vermal Purkinje cells appear to be involved in the regulation of emotional behavior [34, 35], we evaluated exploratory activity in the elevated plus maze (EPM), a test widely used to assess anxiety-like behaviors in mice and rats [1].

Materials and methods

Animals and experimental design

Pregnant CF-1 mice were housed individually in laboratory cages (30×18×12 cm) and maintained under controlled conditions: light (12/12 h), temperature (21±2°C), and humidity (60-70%). Pregnancy was detected by the presence of semen in vaginal smears (gestational day 0, G0). Pregnant dams were randomly assigned to control (n=14) or stressed (n=16) groups and housed individually in standard laboratory cages (47×26×15 cm) with food and water available ad libitum. Maternal stress was induced using a plastic tube (4×11 cm) that fit closely to the body size. The tube was perforated to allow adequate ventilation during the restraint periods. The pregnant dam was gently introduced on the restrainer three times a day (07.00, 13.00, and 19.00 h) for 45 min per session [44], between G14 and G20 [3, 16, 41]. This procedure was chosen because it has been demonstrated to raise corticosterone levels in the blood and, consequently, is an effective stressor [15, 24]. Additionally, the last week of gestation in rodents coincides with the second trimester of gestation in humans, a period of accelerated Purkinje cell dendritogenesis [10, 14]. As stressed dams, control dams were housed individually but left undisturbed in their home cages. After birth, animals of control (n=23) and stressed (n=32) groups were kept with their mothers until the end of lactation period (P21); then were placed socially (four to six animals per cage) in the same room and under the same environmental conditions. In addition, to avoid gender-related influences, the behavioral and neuronal assessments were conducted only in male animals.

Elevated plus maze

All animals were tested on the EPM at P22, P52, and P82. The EPM was constructed of black Plexiglas and consisted of two open arms (60×6 cm) and two closed arms (60×6×14 cm). The device was mounted on a fixed base, 41.5 cm above the floor. Each animal was placed in the center of the EPM and allowed to freely explore the maze for 5 min. Both the time spent in, and the number of entries into the open arms, were recorded and expressed as a percentage of the total time spent in or entries into any of the four arms. The EPM presents the animal with a choice between exploration of novel environments and natural fear of open spaces. Animals that explored the open arms more frequently were considered less anxious compared to animals that remained in the enclosed arms [6]. Moreover, anxiolytic agents have been found to increase entries and time spent in the open arms, while anxiogenic agents elicit the opposite effect [31]. Placement of all four limbs into one arm of the maze was defined as an entrance into that arm. Exploratory behavior was recorded using a webcam (Logitech Quick Cam 9.5.0) situated 30 cm above the EPM. These data were processed with MATLAB 7.0 software.

Purkinje cell morphology

At P22, 52, and 82, the mice were weighed and sacrificed under deep anesthesia with ether. The cerebella were carefully dissected, fixed, and stained in the Golgi-Cox-Sholl solution. After 30 days, the tissue was dehydrated in 50% alcohol-acetone and 50% alcohol-ether, embedded in celoidin, and hardened with chloroform vapors (Merck). Sagittal sections (100–120 μm thick) were obtained from the cerebellar vermis, mounted on slides, cleared with terpineol, covered with Canadian balsam, and coverslipped for light microscopic analysis (Olympus CX-31). Vermal sections were examined in sequence moving in the rostral to caudal direction. The sections analyzed included lobules I-X according to Larsell's terminology. A total of 241 Purkinje cells were analyzed from the following timepoints: P22 (control: n=41, stressed: n=42); P52 (control: n=45, stressed: n=39); P82 (control: n=38, stressed: n=36). To assure consistent and reliable neuronal sampling, all Purkinje cells that were assessed in the study met the

following parameters: homogeneous impregnation, symmetrical dendritic trees, a dendritic arbor parallel to the plane of section, and an absence of dendritic breaks. Selected neurons meeting the above criteria were captured with a digital camera attached to the microscope (Olympus CCD 5.0). The dendritic area (i.e., all dendritic surface/neuron minus the remaining neuropil) was quantified using Micrometrics SE Premium V-2.8 software.

Animals were treated and housed in accordance with the "Principles of Laboratory Animal Care" (NIH publication N° 86-23, revised 1985), and experimental protocols received approval from the local animal ethics committee.

Statistical analysis

Experimental data were statistically analyzed with a one-way ANOVA test. When significant differences ($p<0.05$) were detected, analyses were complemented post hoc with the Scheffé test (STATA 9.1 software).

Results

Figure 1 show percentages of time spent (Fig. 1a) and entries (Fig. 1b) into the open arms; no significant differences were found between experimental groups at the ontogenetic stages evaluated. As shown in Fig. 2, the average dendritic area was initially increased in animals subjected to PS (~29% at P22; ~35% at P52; $p<0.01$). When mice reach P82, however, Purkinje cells showed a dramatic decrease in dendritic material when compared to age-matched controls (~70%; $p<0.01$). In addition, Fig. 3 shows that no significant Purkinje cell growth occurs in postweaning and juvenile animals of both control and PS group. However, from adolescence to P82, the control animals showed a significant dendritic growth in relation to previous ages (Fig. 3a; $F_{(2,\ 69)}$: 12.1; $p<0.01$). By contrast, age-matched PS animals exhibited a dramatic dendritic tree reduction compared to their younger counterparts (Fig. 3b; $F_{(2,\ 56)}$: 30.3; $p<0.01$). Figure 4 shows six representative photomicrographs taken from control (A, C, E) and PS (B, D, F) mice in the three ontogenetic stages studied (A, B: P22; C, D: P52; E, F: P82). Finally, there were no significant differences in body weight at any ontogenetic stage studied.

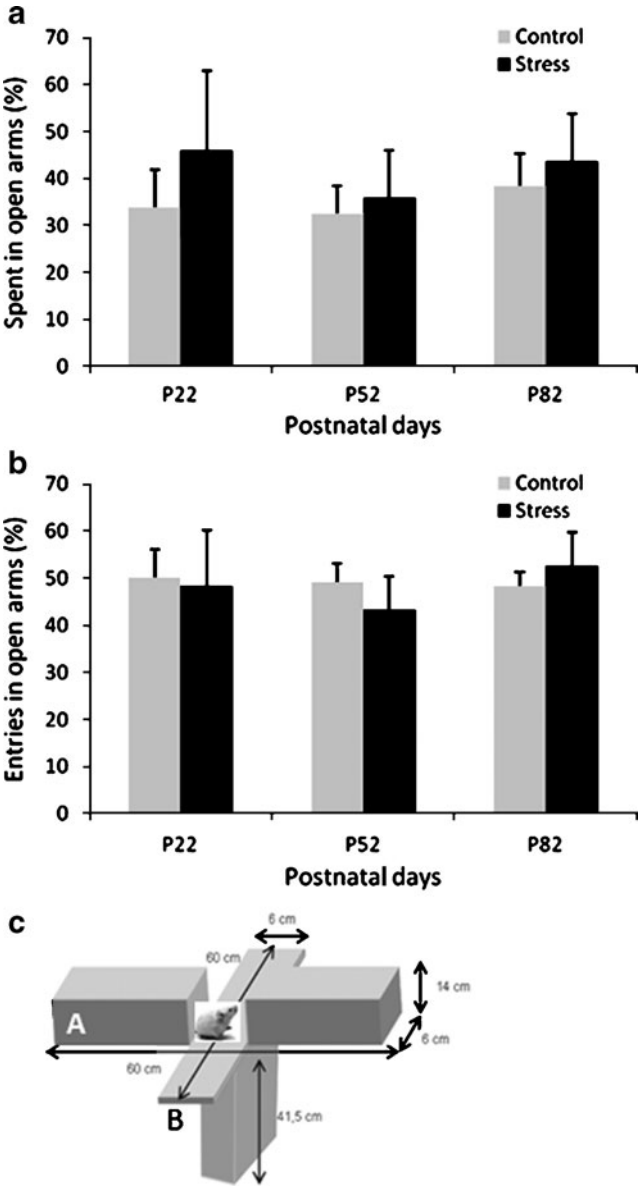

Fig. 1 Percentages of time spent (**a**) and number of entries (**b**) into the open arms (EPM) in control and prenatally stressed mice at juvenile (*P22*), adolescent (*P52*), and adult (*P82*) ages (**c**). EPM, *A* closed arms, *B*, open arms. Data are mean±SEM (ANOVA test)

Discussion

In the current study, we showed that cerebellar Purkinje cells in P22 and P52 animals submitted to PS exhibit significant dendritic overgrowth when compared to age-matched controls (P22=29%; P52=35%). This increased dendritic expansion, however, is transient. Purkinje cells evaluated in P82 showed a dramatic dendritic field reduction (approximately 70%). The bimodal response of Purkinje cells to prenatal stress is very interesting because it is similar to what happens in the brain of children with autism spectrum disorder (ASD). Several studies have reported that the brains of ASD children show a bimodal pattern of growth; during the first 2-3 postnatal years, the brain growth occurs at an above-average rate, and then it exhibits a significant growth arrest [11]. Interestingly, there is an increased incidence of autism in children whose mothers have undergone stressful experiences during

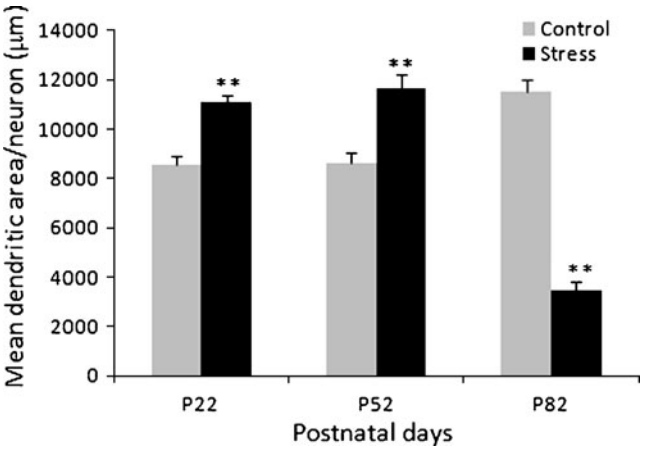

Fig. 2 Purkinje cell area quantified in control and prenatally stressed mice at juvenile (*P22*), adolescent (*P52*), and adult (*P82*) ages. Data are mean±SEM; $^{**}p<0.01$, ANOVA test

pregnancy [5, 21]. Furthermore, several clinical, pathological, and neuroimaging studies using tractography have shown that ASD is associated with a smaller or larger cerebellar vermis [12, 20] and altered cerebellar neural circuitry [9].

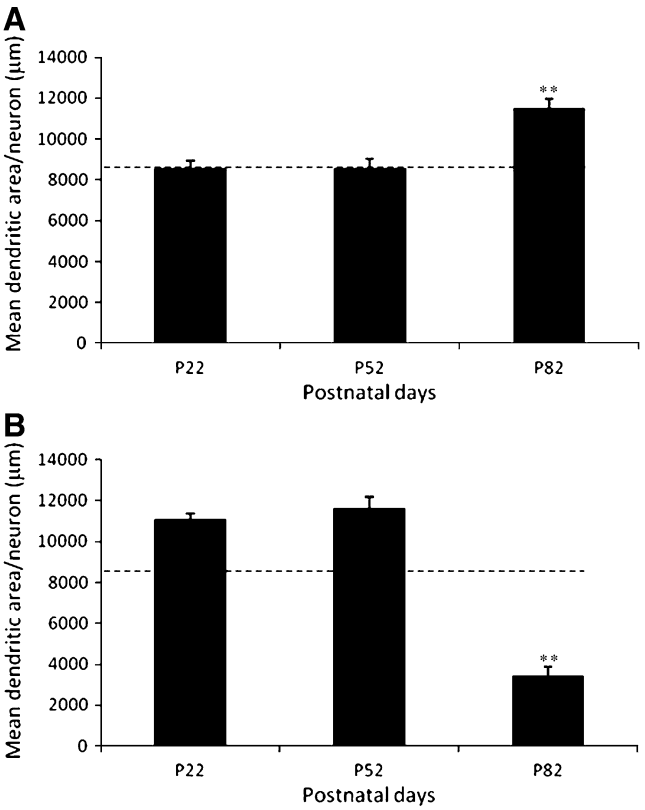

Fig. 3 Time course of dendritic growth in control (**a**) and prenatally-stressed animals (**b**). *P22*, *P52*, and *P82* as in Fig. 2. *Dashed line* highlights the ontogenetic changes in the dendritic tree of Purkinje cells. Data are mean±SEM; $^{**}p<0.01$, ANOVA test

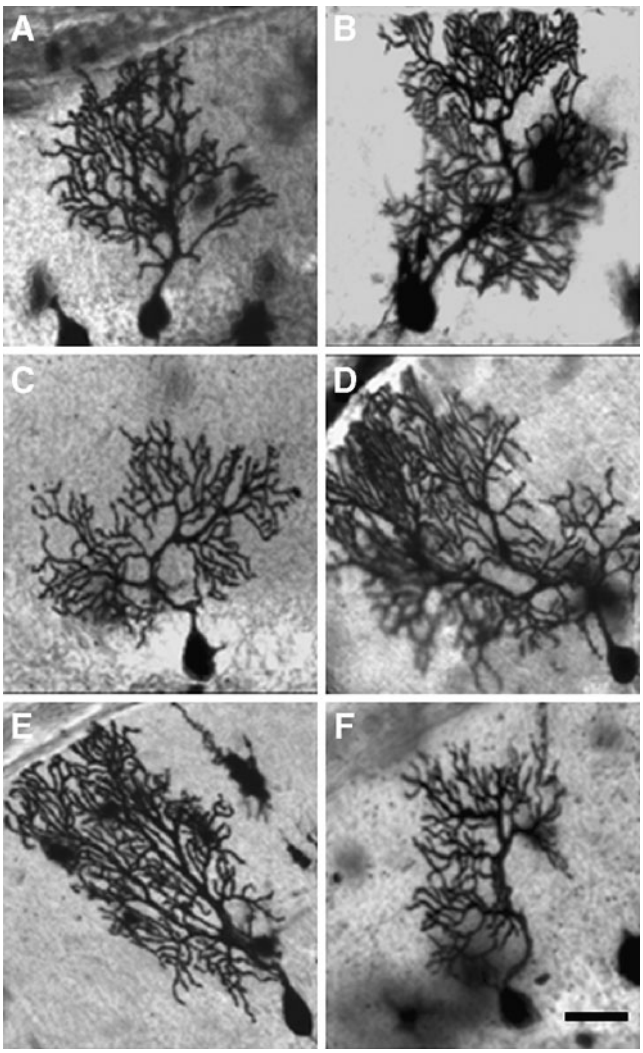

Fig. 4 Representative Purkinje cell photomicrographs taken from control (**a**, **c**, **e**) and prenatally stressed (**b**, **d**, **f**) mice, in the three ontogenetic stages studied (**a**, **b** P22; **c**, **d** P52; **e**, **f** P82). The dendritic fields of juvenile and adolescent stressed mice are qualitatively larger than the age-matched control animals On the contrary, the dendritic trees of adult prenatally stressed animals (P82) are less developed than age-matched controls. *Bar* 40 μm

In the present work, we submitted animals to prenatal stress during the third week of gestation. This period corresponds approximately to the second trimester of gestation in humans [10, 14]. It has been proposed that this ontogenetic stage coincides with cerebral and cerebellar cytoarchitecture formation, thus making the fetus particularly vulnerable to stressors that may result in the development of psychiatric disorders. Interestingly, in a recent MRI study carried out in 6- to 9-year-old children, Buss et al. [8] found a significant association between high anxiety during pregnancy and a decrease in gray matter density in a number of brain regions, including the cerebellum. The fact that these structural changes were found during the second but not the third trimester of

gestation supports the notion that, in humans, the second trimester of gestation is a period of elevated vulnerability to early stressful experiences.

The bimodal ontogenetic Purkinje cell response reported here can be explained, at least in part, by the strong dynamics of dendritic growth and the reorganization that Purkinje cells exhibit during the early postnatal weeks. Morphological studies of Purkinje cell development in rats and mice show that the maturation of dendritic expansions exhibits a remarkable plasticity, characterized by elongation, retraction, and continued remodeling. These changes occur primarily during the first 3-4 weeks of postnatal life [27, 38]. Furthermore, because Purkinje cell dendrites are densely innervated by parallel axons from granule cells and prenatal stress significantly alters the granular cell connectivity [40, 41], it is possible that the observed dendritic tree reduction reported in the present study may be generated indirectly by morphofunctional alterations in cerebellar granular cells following PS. When cerebellar granular cells fail to migrate and die before the elaboration of parallel fibers, as occur in homozygous *weaver* or *staggerer* mutant mouse, the dendritic trees of Purkinje cells are significantly reduced and randomly oriented [7]. These findings suggest that granular cells exert a significant trophic-like effect on the maturation and maintenance of Purkinje cell dendritic trees. In the mouse cerebellum, two important neurotrophins, brain derived neurotrophic factor and neurotrophin 3, are released by granule cells and bind to postsynaptic Purkinje cell dendritic tree receptors, eliciting powerful trophic effects [4, 22].

Also, somewhat enigmatic result was the dendritic overgrowth observed in P22 and P52 animals subjected to PS. Although it is not possible to know what caused this unexpected morphological change, it may be a compensatory neurobiological mechanism to preserve the neuronal cytoarchitecture [26]. Likewise, because during prenatal development glucocorticoids (GCs) may temporarily accelerate the maturation of various tissues, including neural tissue [36], it is likely that the PS-induced raisings in GCs levels may account for the observed overgrowth in Purkinje cells detected in PS animals. Also, dendritic impairment generated by PS may be due to long-lasting hypothalamic-pituitary-adrenal (HPA) axis alterations following prenatal stress. This hypothesis, called "prenatal programming" [2, 19], states that stressful experiences during fetal development induced by chronic maternal emotional disorders make the fetal HPA axis more reactive later in life. This conjecture has received support from studies reporting that administration of endogenous or exogenous GCs has long-lasting effects in the fetal brain, including changes in neuroendocrine activity, neurotransmitter systems, and transcriptional machinery [16, 45]. Thus, it is feasible that the dendritic impairments observed in prenatally stressed adult animals may be related to various epigenetic processes that sensitize the HPA axis, resulting in excessive increases in GCs that may affect Purkinje and other brain cell morphology later in life [17, 19, 42].

On the other hand, as prenatally-stressed animals were raised by their biological mothers, another important variable that can account for the reported Purkinje cell changes are the quality of preweaning mother-pup interaction. Since stress may affect maternal behavior, it is likely that the combined effect of inadequate mothering plus PS have enhanced the neuronal damage reported in this study. In fact, it was recently shown that the deleterious effect of prenatal stress on behavioral development may be minimized when the animals are reared by non-stressed mothers [13]. To address this interesting question, future studies should analyze the impact of PS on Purkinje cell maturation in animals reared by either stressed or controls surrogate mothers (cross-fostering procedure).

Mice submitted to PS did not exhibit behaviors consistent with anxiety disorders. The EPM is one of the most widely used tests for anxiety in rodents, which evaluates the conflict between the motivation to explore novel environments and the potential "risk" that is involved with open spaces. The present results agree with a study by Bogoch et al. [6] that failed to demonstrate anxiogenic behaviors in male but not female rats exposed to a mixture of three different prenatal stressors (i.e., restraint, forced swim, and saline injections) between GD17 and 22 and behaviorally evaluated at 12 weeks of age. However, the opposite was found by Zuena et al. [46] that failed to find anxious behaviors in prenatally stressed female but not male rats. These inconsistencies may be due to variability in the paradigms used in different laboratories, including the species used, type of stressor, period or duration of stressors, and the postnatal period in which the anxious behavior was evaluated.

In conclusion, the present study showed that prenatal stress in postweaning and adolescent male mice results in a transient increase in the Purkinje cell dendritic field followed by a dramatic dendritic atrophy when animals reach adulthood. This result raises the possibility that changes in Purkinje cell phenotype that occur at critical times of development may contribute to the pathophysiology of some neuropsychiatric disorders associated with vermal dysfunctions.

Acknowledgments This research was supported by Grant PUCV-VRIEA 127.706/2008.

Conflict of interests The authors declare that they have no conflicts of interest.

References

1. Andreatini R, Bacellar LFS (2000) Animal models: trait or state measure? The test-retest reliability of the elevated plus-maze and

behavioral despair. Prog Neuropharmacol Biol Psychiat 24:549–560
2. Barker DJ (2002) Fetal programming of coronary heart disease. Trends Endocrinol Metab 13:364–368
3. Barros VG, Duhalde-Vega M, Caltana L et al (2006) Astrocyte-neuron vulnerability to prenatal stress in the adult rat brain. J Neurosci Res 83:787–800
4. Bates B, Rios M, Trumpp A et al (1999) Neurotrophin-3 is required for proper cerebellar development. Nat Neurosci 2:115–117
5. Beversdorf DQ, Manning SE, Hillier A et al (2005) Timing of prenatal stressors and autism. J Autism Dev Disord 35:471–478
6. Bogoch Y, Biala YN, Linial M et al (2007) Anxiety induced by prenatal stress is associated with suppression of hippocampal genes involved in synaptic function. J Neurochem 101:1018–1030
7. Bradley P, Berry M (1978) The Purkinje cell dendritic tree in mutant mouse cerebellum. A quantitative Golgi study of weaver and staggerer mice. Brain Res 142:135–141
8. Buss C, Davies EP, Muftuler LT et al (2010) High pregnancy anxiety during mid-gestation is associated with decreased gray matter density in 6-9-year-old children. Psychoneuroendocrinology 35:141–153
9. Catani M, Jones DK, Daly E et al (2008) Altered cerebellar feedback projections in Asperger syndrome. Neuroimage 41:1184–1191
10. Clancy B, Darlington RB, Finlay BL (2001) Translating developmental time across mammalian species. Neuroscience 105:7–17
11. Courchesne E, Carper R, Akshoomoff N (2003) Evidence of brain overgrowth in the first year of life in autism. JAMA 290:337–344
12. DelBello MP, Strakowski SM, Zimmerman ME et al (1999) MRI analysis of the cerebellum in bipolar disorder: a pilot study. Neuropsychopharmacology 21:63–68
13. Del Cerro MC, Pérez-Laso C, Ortega E et al (2010) Maternal care counteracts behavioral effects of prenatal environmental stress in female rats. Behav Brain Res 208:593–602
14. Dobbing J, Sands J (1979) Comparative aspects of the brain growth spurt. Early Hum Dev 3:79–83
15. Fujioka A, Fujioka T, Ishida Y et al (2006) Differential effects of prenatal stress on the morphological maturation of hippocampal neurons. Neuroscience 141:907–915
16. Hayashi R, Wada H, Ito K et al (2006) Effects of glucocorticoids on gene transcription. Eur J Pharmacol 500:51–62
17. Herbert J, Goodyer IM, Grossman AB et al (2006) Do corticosteroid damage the brain? J Neuroendocrinol 18:393–411
18. Ichimiya T, Okubo Y, Suhara T et al (2001) Reduced volumen of the cerebellar vermis in neuroleptic-naive schizophrenia. Biol Psychiatry 49:20–27
19. Kapoor A, Dunn E, Kostaki A, Andrews MH, Matthews SG (2006) Fetal programming of hypothalamo pituitary-adrenal function: prenatal stress and glucocorticoids. J Physiol 572:31–44
20. Kaufmann WE, Cooper KL, Mostofsky SH et al (2003) Specificity of vermian cerebellar abnormalities in autism. A quantitative magnetic resonance imaging study. J Child Neurol 18:463–470
21. Kinney DK, Munir KM, Crowley DJ et al (2008) Prenatal stress and risk for autism. Neurosci Biobehav Rev 32:1519–1532
22. Lindholm D, Hamner S, Zirrgiebel U (1997) Neurotrophins and cerebellar development. Perspect Dev Neurobiol 5:83–94
23. Loeber RT, Cintron CM, Yurgen-Todd DA (2001) Morphometry of individual cerebellar lobules in schizophrenia. Am J Psychiatry 158:952–954
24. Maccari S, Morley-Fletcher S (2007) Effects of prenatal restraint stress on the hypothalamus-pituitary-adrenal axis and related behavioural and neurobiological alterations. Psychoneuroendocrinology 32:10–15
25. Mackie S, Shaw P, Lenroot R et al (2007) Cerebellar development and clinical outcome in attention deficit hyperactivity disorder. Am J Psychiatry 164:647–655
26. Matthews SG (2001) Antenatal glucocorticoids and the developing brain: mechanisms of action. Semin Neonatol 6:309–317
27. McKay BE, Turner RW (2005) Physiological and morphological development of the rat cerebellar Purkinje cell. J Physiol 567:829–850
28. Onat F, Cavdar S (2003) Cerebellar connections: hypothalamus. Cerebellum 2:263–269
29. Pascual R, Hervias MC, Tohá ME et al (1998) Purkinje cell impairment induced by early movement restriction. Biol Neonate 73:47–51
30. Pascual R, Verdú E, Valero A et al (1999) Early social isolation decreases the expression of calbindin D-28k in rat cerebellar Purkinje cells. Neurosci Lett 272:171–174
31. Pellow S, File SE (1986) Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav 24:525–529
32. Picard H, Amado I, Mouchet-Mages S et al (2008) The role of the cerebellum in schizophrenia: an update of clinical, cognitive, and functional evidences. Schizophr Bull 34:155–172
33. Pysh JJ, Weiss GM (1979) Exercise during development induces an increase in Purkinje cell dendritic tree size. Science 206:230–232
34. Schmahmann JD, Sherman JC (1988) The cerebellar cognitive affective syndrome. Brain 121:561–579
35. Schutter DJ, Van Honk J (2005) The cerebellum on the rise in human emotion. Cerebellum 4:290–294
36. Sousa CJJ, Almeida OFX (2008) Corticosteroid receptors and neuroplasticity. Brain Res Rev 57:561–560
37. Strick PL, Dum RP, Fiez JA (2009) Cerebellum and nonmotor function. Annu Rev Neurosci 32:413–434
38. Tanaka M, Yanagawa Y, Obata K et al (2006) Dendritic morphogenesis of cerebellar Purkinje cells through extension and retraction revealed by long-term tracking of living cells in vitro. Neuroscience 141:663–674
39. Tavano A, Grasso R, Gagliardi Ch et al (2007) Disorders of cognitive and affective development in cerebellar malformations. Brain 130:2646–2660
40. Ulupinar E, Yucel F (2005) Prenatal stress reduces interneuronal connectivity in the rat cerebellar granular layer. Neurotoxicol Teratol 27:475–484
41. Ulupinar E, Yucel F, Ortug G (2006) The effects of prenatal stress on the Purkinje cell neurogenesis. Neuroteratol Toxicol 28:86–94
42. Valleé M, Mayo M, Dellu F et al (1997) Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-Induced corticosterone secretion. J Neurosci 17:2626–2636
43. Wadhwa PD, Culhane JF, Rauh V et al (2001) Stress and preterm birth: neuroendocrine, immune/inflammatory, and vascular mechanisms. Matern Child Health 5:119–125
44. Watson JB, Mednick SA, Huttunen M et al (1999) Prenatal teratogens and the development of adult mental illness. Dev Psychopathol 11:457–466
45. Welberg LAM, Thrivikraman KV, Plotsky PM (2005) Chronic maternal stress inhibits the capacity to up-regulate placental 11β-hydroxysteroid dehydrogenase type 2 activity. J Endocrinol 186:R7–R12
46. Zuena AR, Mairesse J, Casolini P et al (2008) Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats. PLoS One 3:e2170