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REVIEW

Animal Models of Autism: An Epigenetic and Environmental Viewpoint

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Abstract: Autism is a neurodevelopmental disorder of social behavior, which is more common in males than in females. The causes of autism are unknown; there is evidence for a substantial genetic component, but it is likely that a combination of genetic, environmental and epigenetic factors contribute to its complex pathogenesis. Rodent models that mimic the behavioral deficits of autism can be useful tools for dissecting both the etiology and molecular mechanisms. This review discusses animal models of autism generated by prenatal or neonatal environmental challenges, including virus infection and exposure to valproic acid (VPA) or stress. Studies of viral infection models suggest that interleukin-6 can influence fetal development and programming. Prenatal exposure to the histone deacetylase inhibitor VPA has been linked to autism in children, and male VPA-exposed rats exhibit a spectrum of autistic-like behaviors. The experience of prenatal stress produces male-specific behavioral abnormalities in rats. These effects may be mediated by epigenetic modifications such as DNA methylation and histone acetylation resulting in alterations to the transcriptome.

Keywords: autism, environmental factors, epigenetic processes, experimental animal models

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Introduction

Autism is a neurodevelopmental disorder in the category of pervasive developmental disorders, and is characterized by severe and sustained impairment in social interaction, deviance in communication, and patterns of behavior and interest that are restricted or stereotyped. The prevalence is approximately 1.6% in the general population, and there is a 4:1 male:female ratio.[1,2] Although twin studies have provided evidence for a strong genetic component for autism,[3] the underlying genetic determinants are still largely unknown. Additionally, the prevalence of autism appears to be increasing rapidly.[4,5] This explosive increase cannot be explained simply by widening diagnostic categories and increasing professional and public awareness. A complex interaction of genetic and environmental factors, such as chemicals, viral infections, and stresses, is suspected.[6–9] Recently, epigenetic factors have been implicated in the pathogenesis of autism.[10] Epigenetic mechanisms typically involve DNA methylation, histone acetylation, and non-coding RNAs, including microRNAs. Increasing evidence shows that numerous types of chromatin modification, referred to as chromatin remodeling, are widespread in the brain and undergo dynamic regulation in the developing nervous system.[11]

This review describes animal models produced by prenatal or neonatal environmental challenges, including exposure to valproic acid (VPA), inflammatory agents or stresses. Finally, based on the common features of these models, we discuss the potential mechanisms of environmental factor-mediated pathogenesis, especially epigenetic aspects. These models are useful tools towards a better understanding of the complex etiology of autism.

Relevant Behaviors in Animal Models of Autism

Certain criteria have been proposed to improve the validity of animal models with regard to the mental disorders that they are intended to model. Robbins and Sahakian identified three criteria: behavioral similarity (face validity), shared etiology involving similarity of underlying neurobiological mechanisms (construct and etiological validity), and pharmacological similarity (predictive validity).[12] Face validity refers to the phenomenological similarity between the behavior exhibited by the animal model and specific symptoms of the human condition. Table 1 shows the correspondence between the three primary behavioral impairments observed in autism and their relevant behavioral measures in mice and rats.[13] The etiology of autism is unknown, and there is no clear and consistent neuroanatomical abnormality.[14] Therefore, in this review, we describe only the models with face validity.

Viral Infections

Maternal bacterial and viral infections during pregnancy represent a risk factor in several neurodevelopmental disorders.[7,15–18] Additionally, many findings point to immune dysregulation in autism. Higher titers of autoantibodies to brain proteins/antigens have been reported in autistic subjects.[19–21] Similarly, evidence for elevated levels of various pro-inflammatory cytokines, such as interleukin (IL)-6, tumor necrosis factor (TNF)-α and IL-1β has been found in the brain,[22,23] and blood[24,25] of autistic individuals.

Many viruses or immunostimulants, including influenza virus, cytomegalovirus, polyinosinic: polycytidylic acid [poly(I:C)], and bacterial lipopolysaccharide have been used to established animal models of psychiatric and neurodevelopmental disorders such as schizophrenia and autism. Shi et al reported that maternal injection of influenza virus causes deficits in social interaction, prepulse inhibition (PPI), and exploratory behavior in the adult offspring.[26] These behavioral changes in the adult offspring suggest that brain development was altered by perturbation of the fetal environment; however, the virus has not been detected in the brains of neonatal mice born to infected mothers.[26] Smith et al have shown that maternal injection of poly(I:C), which evokes an antiviral-like immune response, causes behavioral deficits in the adult offspring.[27] This indicates that the maternal immune response is sufficient to cause changes in the behavior of the adult offspring. Additionally, Smith et al demonstrated that IL-6 was elevated in poly(I:C) model mice, and co-administration of an anti-IL-6 antibody, but not an anti-TNF-α antibody, prevents the social interaction and PPI deficits caused by poly(I:C) in adult offspring.[27] Similarly, maternal injection of poly(I:C) into IL-6 knockout mice does not effect all of the expected behavioral changes.

Table 1. Clinical aspects of autistic disorder and relevant behaviors in animal models (based on Tordjman et al 2007).

Behavioral impairments	Behavioral measures in mice and rats
Social interaction	Decreased huddle, groom, barber, and play (chasing, sparring, wrestling, pinning) behavior, social exploration (approach, nose grooming), sexual activity (following, sniffing, mounting, genital grooming), aggression (threatening, attacking, biting, *etc.*)
Cognitive and communication behaviors	Decreased pup distress calls, mating calls, submissive calls
Stereotypical behaviors	Increased repeated motor activities (spontaneous activity, exploration, circling, digging, jumping, *etc.*) Increased self-injurious behaviors and other self-involved behaviors (self-grooming, scratching, washing, *etc.*)

Although Borna disease virus (BDV) has not been implicated in the pathogenesis of autism, neonatal BDV infection profoundly affected social behaviors and stereotypic behaviors in adult rats.[28,29] In these rats, glial activation is prominent throughout the brain and persists for several weeks in concert with increased levels of proinflammatory cytokine mRNAs including IL-6 mRNA.

These findings suggest that IL-6 as a key intermediary should aid in the molecular dissection of the pathways whereby maternal immune activation alters brain development. IL-6 induces Janus tyrosine kinase-2/signal transducer and activator of transcription-3 (JAK2/STAT3) phosphorylation, and induction of neuronal JAK2/STAT3 phosphorylation following IL-6 challenge led to significant deficits in social interaction behaviors in mice.[30] Another potential mechanism is related to the inhibitory effect of IL-6 on DNA methylation. It has been reported that IL-6 exerts many epigenetic changes in cells *via* increasing expression of the DNA (cytosine-5-)-methyltransferase 1 gene (*DNMT1*).[31,32] DNMT1 transfers a methyl group to the cytosine portion of the CpG dinucleotide, and permits or enables the binding of methyl-specific DNA-binding proteins to the methylated CpG site. Methylcytosine DNA-binding proteins can attract histone deacetylases to the site, which remodel chromatin into a highly repressed state.[33] Thus, DNA methylation can result in permanent epigenetic alteration of genes.

Valproic Acid Exposure from Medication

Of the environmental agents linked to autism, VPA has been studied the most extensively. Current indications for VPA include: epilepsy,[34,35] mania,[36,37] and migraine prophylaxis.[38] VPA is a recognized teratogen. Exposure *in utero* is associated with low myelomeningocele lesions, abnormalities of the face, and occasional major organ abnormalities involving the respiratory, cardiovascular, gastrointestinal, genitourinary and skeletal systems.[39,40] The possible association between *in utero* VPA exposure and autism was first reported by Christianson et al in 1994.[42] These authors described four children exposed *in utero* to VPA: all demonstrated developmental delays and one of these children also had autism. Many reports appeared thereafter in the literature associating VPA exposure with autism.[43–49] According to Rasalam et al the rate of autism, including pervasive developmental disorder and Asperger syndrome, may be 20 times higher in VPA-exposed children than the expected rate in the general population.[50]

Rodier et al developed an animal model of autism by exposing rats to VPA *in utero* (the VPA rat).[51] Thereafter, several researchers have examined the behavior of these rats, and showed that VPA rats exhibit impaired social interaction, increased repetitive/stereotypic-like activity, increased nociceptive thresholds, enhanced anxiety, and increased fear memory.[52–54] Interestingly, these behavioral alterations are gender-specific. Male VPA rats show the autistic-like behaviors as described above, while female VPA rats exhibit only increased repetitive/stereotypic-like activity. Because autistic patients show various abnormalities in the immune system, Schneider et al also measured the immune parameters of VPA rats. Male VPA rats exhibit increased basal levels of corticosterone, decreased weight of the thymus, a decreased splenocyte proliferative response to concanavalin A, a lower interferon (IFN)-γ/IL-10 ratio, and increased production of nitric oxide by peritoneal macrophages. On the other hand, female VPA rats displayed only a decreased IFN-γ/IL-10 ratio. These results confirm

the similarities between the aberrations in VPA rats and the disturbed behavior and immune function in autistic patients. The rat phenotypes also appear to be gender-specific, which is intriguing in light of the disproportionate male to female ratio in autism. Although Rasalam et al found more female VPA-exposed fetuses displaying features of autism, the sample size in this study was very small (two males, three females).[50] Additional research is needed to investigate the male to female ratio in autism caused by VPA exposure.

Recently, epigenetic factors have been implicated in the pathogenesis of autism. It has been reported that VPA inhibits histone deacetylase (HDAC).[55] HDAC reduces the acetylation of histones, inducing chromatin changes that affect the interaction of transcription factors and RNA polymerase with DNA, thereby modulating gene transcription. Inhibition of HDAC has been estimated to cause approximately 2% of transcriptionally inactive genes to become available for transcription *via* its effect on chromatin.[56] In *Xenopus laevis* and zebrafish embryos, VPA induced growth retardation and a variety of congenital anomalies, all resulting from HDAC inhibition.[57] Additionally, the acetylation of H3 histones that results from the direct action of VPA increases the accessibility of demethylases to DNA, resulting in active demethylation. Indeed, VPA treatment changes the expression of various genes including *Bcl2* and *Hoxa1*, and activates Wnt-dependent gene expression.[58–61] These studies may help to explain the brain damage in the offspring of women treated with VPA during pregnancy.

VPA also has anti-folate activity. Reduced embryonic folic acid may disrupt gene expression, increase embryonic oxidative stress and induce changes in protein synthesis.[62,63] Additionally, a polymorphism in the methylenetetrahydrofolate (*MTHFR*) gene, which encodes an important enzyme in the folic acid metabolic pathway, has been suggested to be associated with autism.[64] However, Kini et al reported that the rate of congenital anomalies was higher in the offspring of mothers with the *MTHFR* polymorphism, and that the effect of VPA was greater than the effect of the polymorphism. These results suggest that the effect of VPA is stronger and that many of the anomalies are induced by a different-VPA-mediated mechanism.[49,65] Increased oxidative stress by intermediate metabolites of VPA has also been suggested.[66,67] Increased oxidative stress was indeed demonstrated in young children treated with VPA.[68] However, there seems to be no direct proof that VPA induces embryonic or fetal oxidative stress.

Stress

Numerous studies have demonstrated that stress may be an important factor in autism. The experience of stress during gestation is associated with an increased incidence of autism.[8,9] Additionally, it has been shown that stress exacerbates autistic behaviors and that these effects may be due to abnormal regulation of the hypothalamic-pituitary axis (HPA) and stress hormones.[69,70]

Prenatal stress produces male-specific decreased social interaction in rats.[71] Additionally, impaired spatial learning and reversal learning have been reported in male offspring exposed to stress.[72,73] On the other hand, prenatally stressed females exhibited increased social interaction and spatial learning.[71,73,74] These findings too are intriguing in light of the disproportionate male: female ratio in autism. Mueller and Bale have suggested that one of the mechanisms underlying male vulnerability may involve sex-specific placental responsivity; stress early in pregnancy significantly increased expression of peroxisome proliferator-activated receptor α (*PPARα*) and insulin-like growth factor binding protein 1 (*IGFBP1*) in male placentas, but decreased the expression of these genes in female placentas.[73] Because stress hormone glucocorticoids increase expression of PPARα,[75] and in turn PPARα increases expression of IGFBP1,[76] the findings of Mueller and Bale support a potential mechanism whereby maternal stress could directly affect placental gene expression. These authors also reported that long-term alterations in the expression of the central stress components, increased corticotropin-releasing factor (CRF) and decreased glucocorticoid receptor (GR), as well as increased HPA responsivity, were present in male offspring. Changes in CRF and GR gene methylation correlated with altered gene expression, providing important evidence for epigenetic programming during early prenatal stress.[77] Poor maternal care or a one-off stressful experience for the neonates led to decreased expression of GR mRNA in the neonates, induced by increased methylation of the nerve growth factor-inducible protein A (NGFI-A) transcription

factor response element located within the *GR* promoter.[77] Methylation of the *GR* promoter prevented binding of NGFI-A, thereby reducing GR gene transcription, an effect that was found to persist into adulthood. The adult offspring were more anxious, and had an attenuated corticosterone response to stress.[78,79] Stresses also alter the immune system. Stresses induce cytokine activity in the brain, pituitary and periphery, and this may underlie the disruption of the cytokine balance in pregnancy.[80,81] Multiple and complex interactions in the neuroendocrine immune system may be involved in stress-induced fetal programming.

Conclusions

Thus far, a single gene responsible for autism has not been identified, and it seems unlikely that any single gene could fully explain the pathogenesis of this complex disorder. Genes transcription is highly regulated and responsive to environmental factors.

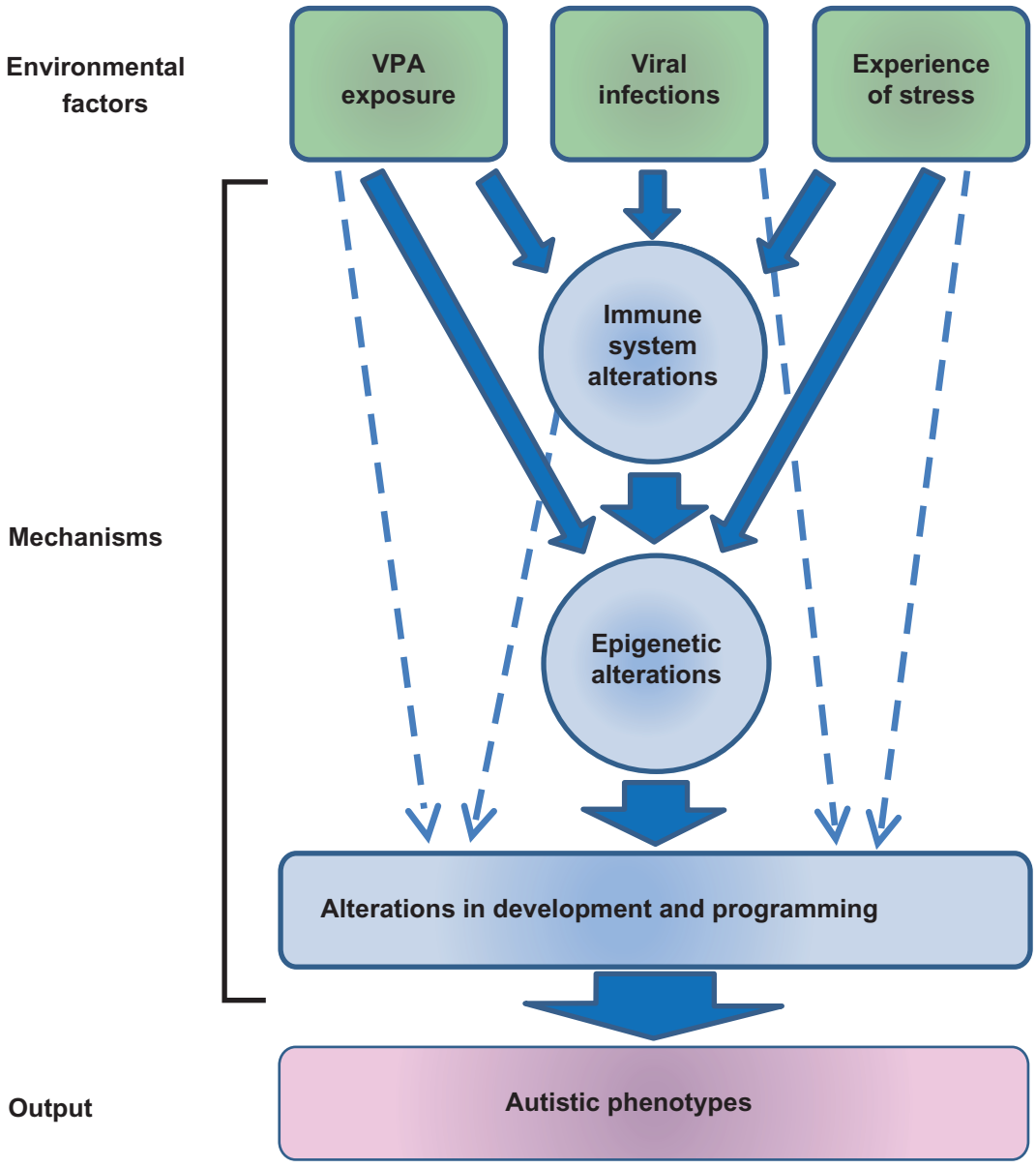

Figure 1. Possible pathogenic mechanisms of autism by environmental factors.
Notes: Viral infections induce immune system alterations, and these alterations lead to epigenetic changes. Valproic acid exposure leads to epigenetic alterations directly or *via* immune system alterations. The experience of stress also can alter epigenetic events or the immune system. Epigenetic alterations cause changes in development and programming of the brain. These changes may ultimately output as an autistic phenotype. Other possible mechanisms are shown as dotted lines.

Thus, animal models generated by exposure to environmental factors are important tools for understanding the pathogenic mechanisms of autism. This review describes animal models produced by prenatal environmental challenges, including exposure to inflammatory agents, VPA and stress. Studies of viral infection models suggest that further investigation of IL-6 should aid in the molecular dissection of the pathways whereby maternal immune activation alters brain development. Intriguingly, IL-6 can influence fetal development and programming through epigenetics (Fig. 1). VPA and stress can also alter fetal development and programming in the same way (Fig. 1). Using this evidence, autistic phenotypes produced by environmental factors possibly result from changes in gene expression, mediated by epigenetic changes including DNA methylation and histone acetylation (Fig. 1). Despite the strongly skewed male:female ratio in autism, no evidence for X-linked loci or a simple sex-limited multifactorial threshold model has been found in twin and family studies.[1] However, gender-specific abnormalities have been demonstrated in VPA rats and stressed rats. These models will be useful in the further understanding of the complex etiology of autism.

Abbreviations

VPA, valproic acid; IL, interleukin; TNF, tumor necrosis factor; poly(I:C), polyinosinic: polycytidylic acid; PPI, prepulse inhibition; BDV, Borna disease virus; JAK2/STAT3, Janus tyrosine kinase 2/signal transducer and activator of transcription 3; DNMT1, DNA (cytosine-5-)-methyltransferase 1; IFN, interferon; HDAC, histone deacetylase; HPA, hypothalamic-pituitary axis; PPARα, peroxisome proliferator-activated receptor α; IGFBP1, insulin-like growth factor binding protein 1; CRF, corticotropin-releasing factor; GR, glucocorticoid receptor; NGFI-A, nerve growth factor-inducible protein A.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

References

1. Freitag C. The genetics of autistic disorders and its clinical relevance: a review of the literature. *Mol Psychiatry*. 2007;12(1):2–22.
2. Baron-Cohen S, Scott F, Allison C, Williams J, Bolton P, Matthews F, et al. Prevalence of autism-spectrum conditions: UK school-based population study. *Br J Psychiatry*. 2009;194(6):500–9.
3. Folstein S, Rosen-Sheidley B. Genetics of autism: complex aetiology for a heterogeneous disorder. *Nat Rev Genet*. 2001;2(12):943–55.
4. Muhle R, Trentacoste S, Rapin I. The genetics of autism. *Pediatrics*. 2004; 113(5):e472–86.
5. Honda H, Shimizu Y, Imai M, Nitto Y. Cumulative incidence of childhood autism: a total population study of better accuracy and precision. *Dev Med Child Neurol*. 2005;47(1):10–8.
6. Geier D, Kern J, Garver C, Adams J, Audhya T, Nataf R, et al. Biomarkers of environmental toxicity and susceptibility in autism. *J Neurol Sci*. 2009;280(1–2):101–8.
7. Libbey J, Sweeten T, McMahon W, Fujinami R. Autistic disorder and viral infections. *J Neurovirol*. 2005;11(1):1–10.
8. Kinney D, Munir K, Crowley D, Miller A. Prenatal stress and risk for autism. *Neurosci Biobehav Rev*. 2008;32(8):1519–32.
9. Gillott A, Standen P. Levels of anxiety and sources of stress in adults with autism. *J Intellect Disabil*. 2007;11(4):359–70.
10. Grafodatskaya D, Chung B, Szatmari P, Weksberg R. Autism spectrum disorders and epigenetics. *J Am Acad Child Adolesc Psychiatry*. 2010;49(8): 794–809.
11. Tsankova N, Renthal W, Kumar A, Nestler E. Epigenetic regulation in psychiatric disorders. *Nat Rev Neurosci*. 2007;8(5):355–67.
12. Robbins T, Sahakian B. "Paradoxical" effects of psychomotor stimulant drugs in hyperactive children from the standpoint of behavioural pharmacology. *Neuropharmacology*. 1979;18(12):931–50.
13. Tordjman S, Drapier D, Bonnot O, Graignic R, Fortes S, Cohen D, et al. Animal models relevant to schizophrenia and autism: validity and limitations. *Behav Genet*. 2007;37(1):61–78.
14. Amaral D, Schumann C, Nordahl C. Neuroanatomy of autism. *Trends Neurosci*. 2008;31(3):137–45.
15. Ciaranello A, Ciaranello R. The neurobiology of infantile autism. *Annu Rev Neurosci*. 1995;18:101–28.
16. Sweeten T, Posey D, McDougle C. Brief report: autistic disorder in three children with cytomegalovirus infection. *J Autism Dev Disord*. 2004; 34(5):583–6.
17. Yamashita Y, Fujimoto C, Nakajima E, Isagai T, Matsuishi T. Possible association between congenital cytomegalovirus infection and autistic disorder. *J Autism Dev Disord*. 2003;33(4):455–9.
18. Patterson P. Maternal infection: window on neuroimmune interactions in fetal brain development and mental illness. *Curr Opin Neurobiol*. 2002; 12(1):115–8.
19. Connolly A, Chez M, Pestronk A, Arnold S, Mehta S, Deuel R. Serum autoantibodies to brain in Landau-Kleffner variant, autism, and other neurologic disorders. *J Pediatr*. 1999;134(5):607–13.
20. Singh V, Rivas W. Prevalence of serum antibodies to caudate nucleus in autistic children. *Neurosci Lett*. 2004;355(1–2):53–6.
21. Singh V, Warren R, Odell J, Warren W, Cole P. Antibodies to myelin basic protein in children with autistic behavior. *Brain Behav Immun*. 1993; 7(1):97–103.
22. Li X, Chauhan A, Sheikh A, Patil S, Chauhan V, Li X, et al. Elevated immune response in the brain of autistic patients. *J Neuroimmunol*. 2009;207(1–2):111–6.
23. Vargas D, Nascimbene C, Krishnan C, Zimmerman A, Pardo C. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol*. 2005;57(1):67–81.
24. Gupta S, Aggarwal S, Rashanravan B, Lee T. Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. *J Neuroimmunol*. 1998;85(1):106–9.
25. Jyonouchi H, Sun S, Le H. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *J Neuroimmunol*. 2001;120(1–2):170–9.

26. Shi L, Fatemi S, Sidwell R, Patterson P. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. *J Neurosci*. 2003;23(1):297–302.
27. Smith S, Li J, Garbett K, Mirnics K, Patterson P. Maternal immune activation alters fetal brain development through interleukin-6. *J Neurosci*. 2007;27(40):10695–702.
28. Hornig M, Weissenböck H, Horscroft N, Lipkin W. An infection-based model of neurodevelopmental damage. *Proc Natl Acad Sci U S A*. 1999; 96(21):12102–7.
29. Lancaster K, Dietz D, Moran T, Pletnikov M. Abnormal social behaviors in young and adult rats neonatally infected with Borna disease virus. *Behav Brain Res*. 2007;176(1):141–8.
30. Parker-Athill E, Luo D, Bailey A, Giunta B, Tian J, Shytle R, et al. Flavonoids, a prenatal prophylaxis via targeting JAK2/STAT3 signaling to oppose IL-6/MIA associated autism. *J Neuroimmunol*. 2009;217(1–2):20–7.
31. Hodge D, Xiao W, Clausen P, Heidecker G, Szyf M, Farrar W. Interleukin-6 regulation of the human DNA methyltransferase (HDNMT) gene in human erythroleukemia cells. *J Biol Chem*. 2001;276(43):39508–11.
32. Foran E, Garrity-Park M, Mureau C, Newell J, Smyrk T, Limburg P, et al. Upregulation of DNA methyltransferase-mediated gene silencing, anchorage-independent growth, and migration of colon cancer cells by interleukin-6. *Mol Cancer Res*. 2010;8(4):471–81.
33. Robertson K, Ait-Si-Ali S, Yokochi T, Wade P, Jones P, Wolffe A. DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. *Nat Genet*. 2000;25(3):338–42.
34. Rimmer E, Richens A. An update on sodium valproate. *Pharmacotherapy*. 1985;5(3):171–84.
35. Beydoun A, Sackellares J, Shu V. Safety and efficacy of divalproex sodium monotherapy in partial epilepsy: a double-blind, concentration-response design clinical trial. Depakote Monotherapy for Partial Seizures Study Group. *Neurology*. 1997;48(1):182–8.
36. Puzyński S, Kłosiewicz L. Valproic acid amide in the treatment of affective and schizoaffective disorders. *J Affect Disord*. 1984;6(1):115–21.
37. Kmetz G, McElroy S, Collins D. Response of kleptomania and mixed mania to valproate. *Am J Psychiatry*. 1997;154(4):580–1.
38. Freitag F, Collins S, Carlson H, Goldstein J, Saper J, Silberstein S, et al. A randomized trial of divalproex sodium extended-release tablets in migraine prophylaxis. *Neurology*. 2002;58(11):1652–9.
39. Robert E, Rosa F. Valproate and birth defects. *Lancet*. 1983;2(8359):1142.
40. DiLiberti J, Farndon P, Dennis N, Curry C. The fetal valproate syndrome. *Am J Med Genet*. 1984;19(3):473–81.
41. Clayton-Smith J, Donnai D. Fetal valproate syndrome. *J Med Genet*. 1995;32(9):724–7.
42. Christianson A, Chesler N, Kromberg J. Fetal valproate syndrome: clinical and neuro-developmental features in two sibling pairs. *Dev Med Child Neurol*. 1994;36(4):361–9.
43. Williams P, Hersh J. A male with fetal valproate syndrome and autism. *Dev Med Child Neurol*. 1997;39(9):632–4.
44. Moore S, Turnpenny P, Quinn A, Glover S, Lloyd D, Montgomery T, et al. A clinical study of 57 children with fetal anticonvulsant syndromes. *J Med Genet*. 2000;37(7):489–97.
45. Dean J, Hailey H, Moore S, Lloyd D, Turnpenny P, Little J. Long term health and neurodevelopment in children exposed to antiepileptic drugs before birth. *J Med Genet*. 2002;39(4):251–9.
46. Viinikainen K, Eriksson K, Mönkkönen A, Aikiä M, Nieminen P, Heinonen S, et al. The effects of valproate exposure in utero on behavior and the need for educational support in school-aged children. *Epilepsy Behav*. 2006;9(4):636–40.
47. Adab N, Kini U, Vinten J, Ayres J, Baker G, Clayton-Smith J, et al. The longer term outcome of children born to mothers with epilepsy. *J Neurol Neurosurg Psychiatry*. 2004;75(11):1575–83.
48. Williams G, King J, Cunningham M, Stephan M, Kerr B, Hersh J. Fetal valproate syndrome and autism: additional evidence of an association. *Dev Med Child Neurol*. 2001;43(3):202–6.
49. Ornoy A. Valproic acid in pregnancy: how much are we endangering the embryo and fetus? *Reprod Toxicol*. 2009;28(1):1–10.
50. Rasalam A, Hailey H, Williams J, Moore S, Turnpenny P, Lloyd D, et al. Characteristics of fetal anticonvulsant syndrome associated autistic disorder. *Dev Med Child Neurol*. 2005;47(8):551–5.
51. Rodier P, Ingram J, Tisdale B, Nelson S, Romano J. Embryological origin for autism: developmental anomalies of the cranial nerve motor nuclei. *J Comp Neurol*. 1996;370(2):247–61.
52. Markram K, Rinaldi T, La Mendola D, Sandi C, Markram H. Abnormal fear conditioning and amygdala processing in an animal model of autism. *Neuropsychopharmacology*. 2008;33(4):901–12.
53. Schneider T, Przewłocki R. Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. *Neuropsychopharmacology*. 2005;30(1):80–9.
54. Schneider T, Roman A, Basta-Kaim A, Kubera M, Budziszewska B, Schneider K, et al. Gender-specific behavioral and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. *Psychoneuroendocrinology*. 2008;33(6):728–40.
55. Phiel C, Zhang F, Huang E, Guenther M, Lazar M, Klein P. Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. *J Biol Chem*. 2001;276(39):36734–41.
56. van Lint C, Emiliani S, Verdin E. The expression of a small fraction of cellular genes is changed in response to histone hyperacetylation. *Gene Expr*. 1996;5(4–5):245–53.
57. Gurvich N, Berman M, Wittner B, Gentleman R, Klein P, Green J. Association of valproate-induced teratogenesis with histone deacetylase inhibition in vivo. *FASEB J*. 2005;19(9):1166–8.
58. Wiltse J. Mode of action: inhibition of histone deacetylase, altering WNT-dependent gene expression, and regulation of beta-catenin—developmental effects of valproic acid. *Crit Rev Toxicol*. 2005;35(8–9): 727–38.
59. Stodgell C, Ingram J, O'Bara M, Tisdale B, Nau H, Rodier P. Induction of the homeotic gene Hoxa1 through valproic acid's teratogenic mechanism of action. *Neurotoxicol Teratol*. 2006;28(5):617–24.
60. Menegola E, di Renzo F, Broccia M, Giavini E. Inhibition of histone deacetylase as a new mechanism of teratogenesis. *Birth Defects Res C Embryo Today*. 2006;78(4):345–53.
61. Chen P, Wang C, Bortner C, Peng G, Wu X, Pang H, et al. Valproic acid and other histone deacetylase inhibitors induce microglial apoptosis and attenuate lipopolysaccharide-induced dopaminergic neurotoxicity. *Neuroscience*. 2007;149(1):203–12.
62. Wegner C, Nau H. Diurnal variation of folate concentrations in mouse embryo and plasma: the protective effect of folinic acid on valproic-acid-induced teratogenicity is time dependent. *Reprod Toxicol*. 1991; 5(6):465–71.
63. Wegner C, Nau H. Alteration of embryonic folate metabolism by valproic acid during organogenesis: implications for mechanism of teratogenesis. *Neurology*. 1992;42(4 Suppl 5):17–24.
64. Boris M, Goldblatt A, Galanko JS, Jill J. Association of MTHFR gene variants with autism. *J Am Phys Surg*. 2004;9(4):106–8.
65. Kini U, Lee R, Jones A, Smith S, Ramsden S, Fryer A, et al. Influence of the MTHFR genotype on the rate of malformations following exposure to antiepileptic drugs in utero. *Eur J Med Genet*. 2007;50(6):411–20.
66. Ornoy A. Embryonic oxidative stress as a mechanism of teratogenesis with special emphasis on diabetic embryopathy. *Reprod Toxicol*. 2007; 24(1):31–41.
67. Danielsson B, Danielsson C, Nilsson M. Embryonic cardiac arrhythmia and generation of reactive oxygen species: common teratogenic mechanism for IKr blocking drugs. *Reprod Toxicol*. 2007;24(1):42–56.
68. Verrotti A, Scardapane A, Franzoni E, Manco R, Chiarelli F. Increased oxidative stress in epileptic children treated with valproic acid. *Epilepsy Res*. 2008;78(2–3):171–7.
69. Tani P, Lindberg N, Matto V, Appelberg B, Nieminen-von Wendt T, von Wendt L, et al. Higher plasma ACTH levels in adults with Asperger syndrome. *J Psychosom Res*. 2005;58(6):533–6.
70. Tordjman S, Anderson G, McBride P, Hertzig M, Snow M, Hall L, et al. Plasma beta-endorphin, adrenocorticotropin hormone, and cortisol in autism. *J Child Psychol Psychiatry*. 1997;38(6):705–15.

71. Ohkawa T. [Sexual differentiation of social play and copulatory behavior in prenatally stressed male and female offspring of the rat: the influence of simultaneous treatment by tyrosine during exposure to prenatal stress]. *Nippon Naibunpi Gakkai Zasshi*. 1987;63(7):823–35.
72. Szuran T, Pliska V, Pokorny J, Welzl H. Prenatal stress in rats: effects on plasma corticosterone, hippocampal glucocorticoid receptors, and maze performance. *Physiol Behav*. 2000;71(3–4):353–62.
73. Mueller B, Bale T. Early prenatal stress impact on coping strategies and learning performance is sex dependent. *Physiol Behav*. 2007;91(1):55–65.
74. Bowman R, MacLusky N, Sarmiento Y, Frankfurt M, Gordon M, Luine V. Sexually dimorphic effects of prenatal stress on cognition, hormonal responses, and central neurotransmitters. *Endocrinology*. 2004;145(8):3778–87.
75. Lemberger T, Staels B, Saladin R, Desvergne B, Auwerx J, Wahli W. Regulation of the peroxisome proliferator-activated receptor alpha gene by glucocorticoids. *J Biol Chem*. 1994;269(40):24527–30.
76. Degenhardt T, Matilainen M, Herzig K, Dunlop T, Carlberg C. The insulin-like growth factor-binding protein 1 gene is a primary target of peroxisome proliferator-activated receptors. *J Biol Chem*. 2006;281(51):39607–19.
77. Weaver I, Cervoni N, Champagne F, D'Alessio A, Sharma S, Seckl J, et al. Epigenetic programming by maternal behavior. *Nat Neurosci*. 2004;7(8):847–54.
78. Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky P, Meaney M. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proc Natl Acad Sci U S A*. 1998;95(9):5335–40.
79. Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*. 1997;277(5332):1659–62.
80. Blois S, Joachim R, Kandil J, Margni R, Tometten M, Klapp B, et al. Depletion of CD8+ cells abolishes the pregnancy protective effect of progesterone substitution with dydrogesterone in mice by altering the Th1/Th2 cytokine profile. *J Immunol*. 2004;172(10):5893–9.
81. Parker V, Douglas A. Stress in early pregnancy: maternal neuro-endocrine-immune responses and effects. *J Reprod Immunol*. 2010;85(1):86–92.

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