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
Introduction
Autism is a neurodevelopmental disorder in the category of pervasive developmental disorders, and is characterized by severe and sustained impairment in social interaction, deviancy 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. Although twin studies have provided evidence for a strong genetic component for autism, the underlying genetic determinants are still largely unknown. Additionally, the prevalence of autism appears to be increasing rapidly. 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. Recently, epigenetic factors have been implicated in the pathogenesis of autism. 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.

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). 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. The etiology of autism is unknown, and there is no clear and consistent neuroanatomical abnormality. 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. Additionally, many findings point to immune dysregulation in autism. Higher titers of autoantibodies to brain proteins/antigens have been reported in autistic subjects. 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 and blood of autistic individuals.

Many viruses or immunostimulants, including influenza virus, cytomegalovirus, polyinosinic: polycytidylic acid [poly(I:C)], and bacterial lipopolysaccharide have been used to establish 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. 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. 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. 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. Similarly, maternal injection of poly(I:C) into IL-6 knockout mice does not affect all of the expected behavioral changes.
Environmental causes of autism

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. 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. 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). 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. 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, mania, and migraine prophylaxis. 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. The possible association between in utero VPA exposure and autism was first reported by Christianson et al in 1994. 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. 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.

Rodier et al developed an animal model of autism by exposing rats to VPA in utero (the VPA rat). 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. Interestingly, these behavioral alterations are gender-specific. Male VPA rats show the autistlike 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). 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). 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. In Xenopus laevis and zebrafish embryos, VPA induced growth retardation and a variety of congenital anomalies, all resulting from HDAC inhibition. 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. 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. 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. However, Kini et al reported that the rate of congenital anomalies was higher in the offspring of mothers taking folic acid. 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. Increased oxidative stress by intermediate metabolites of VPA has also been suggested. Increased oxidative stress was indeed demonstrated in young children treated with VPA. 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. 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.

Prenatal stress produces male-specific decreased social interaction in rats. Additionally, impaired spatial learning and reversal learning have been reported in male offspring exposed to stress. On the other hand, prenatally stressed females exhibited increased social interaction and spatial learning. 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. Because stress hormone glucocorticoids increase expression of PPARα, and in turn PPARα increases expression of IGFBP1, 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. 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. 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. 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. 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.

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**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. 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.

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