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Author
Lein, Pamela J

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Overview of the Role of Environmental Factors in Neurodevelopmental Disorders

Running title: Overview: Neurodevelopmental Disorders

Abstract

Evidence implicates environmental factors in the pathogenesis of diverse complex neurodevelopmental disorders. However, the identity of specific environmental chemicals that confer risk for these disorders, and the mechanisms by which environmental chemicals interact with genetic susceptibilities to influence adverse neurodevelopmental outcomes remain significant gaps in our understanding of the etiology of most neurodevelopmental disorders. It is likely that many environmental chemicals contribute to the etiology of neurodevelopmental disorders but their influence depends on the genetic substrate of the individual. Research into the pathophysiology and genetics of neurodevelopmental disorders may inform the identification of environmental susceptibility factors that promote adverse outcomes in brain development. Conversely, understanding how low level chemical exposures influence molecular, cellular and behavioral outcomes relevant to neurodevelopmental disorders will provide insight regarding gene-environment interactions and possibly yield novel intervention strategies.

Keywords: Autism, ADHD, environmental risk factors, gene-environment interactions, genetic susceptibility, neurodevelopmental disorders, synaptic connectivity

Introduction

Neurodevelopmental disabilities, including autism spectrum disorders (ASD), attention-deficit hyperactivity disorder (ADHD), schizophrenia, learning disabilities, intellectual disability (also known as mental retardation) and sensory impairments, affect 10-15% of all births in the United States (1,2). ADHD and ASD are among the most common of the neurodevelopmental disorders. In 2007, the worldwide prevalence of ADHD was estimated to be 5.3% of children and adolescents (3), and this prevalence is thought to be increasing worldwide (2). The prevalence of ASD has increased dramatically from an estimated 1:150 children reported by the United States Center for Disease Control (CDC) in 2007 based on 2000 and 2002 data to the current estimate of 1:68 children or 1:42 boys (4). Subclinical decrements in brain function are even more common than either of these neurodevelopmental disorders (5). When considered in the context of the tremendous costs that these disorders and disabilities exact on the affected individual, their families and society (6-8), these statistics of their prevalence underscore the urgent need to identify factors that confer risk for neurodevelopmental disabilities.

Until recently, research on the etiology of neurodevelopmental disorders has focused largely on genetic causes (2,9,10). However, this research has clearly shown that single genetic anomalies can account for only a small proportion of cases (2,11) and that overall, genetic factors seem to account for at most 30-40% of all cases of neurodevelopmental disorders (5). There is now credible evidence that many neurodevelopmental disorders are the result of complex gene-environment interactions (11-15). In contrast to genetic risks, which are currently irreversible, environmental factors are modifiable risk factors. Therefore, identifying specific environmental factors that increase risk for neurodevelopmental disorders may

provide rational approaches for the primary prevention of the symptoms associated with these disorders. However, to date, the identities of environmental factors that influence susceptibility to and/or severity of neurodevelopmental disorders and intellectual disabilities, and the mechanisms by which environmental factors interact with genetic factors to determine individual risk, remain critical gaps in our understanding. This chapter will provide an overview of the evidence implicating environmental factors in determining risk of neurodevelopmental disorders, using autism and ADHD as prime examples. This chapter will also briefly discuss proposed mechanisms by which environmental factors might influence risk, and summarize the challenges and opportunities in this important area of research.

Evidence implicating environmental factors

The most compelling evidence in support of the hypothesis that environmental factors contribute to risk of neurodevelopmental disorders is the rapid increase in the prevalence of ASD and ADHD over the past several decades (2), which seems unlikely to have been caused entirely by significant shifts in the human genome. Concerns have been raised as to how much of the increased prevalence of these disorders represents true increases in the numbers of affected children. It has been suggested that diagnostic substitution, e.g., the labeling of individuals as autistic or ADHD who previously would not have been so labeled, as a result of broadening of diagnostic criteria, coupled with increased awareness and improved detection of these disorders, explain their increased prevalence (16-18). However, several studies that have investigated these concerns in the context of ASD concluded that there is indeed a true increase in the frequency of this disorder,

with factors other than broadening of diagnostic criteria and increased awareness likely accounting for more than half of all new cases (19-21).

Studies of the genetic causes of neurodevelopmental disorders also support a role for environmental factors in determining the risk for many neurodevelopmental deficits. Autism is considered to be one of the most heritable complex neurodevelopmental disorders (22,23); however, genes linked to ASD rarely segregate in a simple Mendelian manner (22). This wide-spread observation has led many to posit that multiple genetic etiologies, including rare, private (*de novo*) single gene mutations that are highly penetrant, inherited common functional variants of multiple genes with small to moderate effects on ASD, or copy number variation (CNV), occur in combination to determine ASD risk (24-28). An alternative interpretation of the genetic findings is that environmental factors act as risk modifiers (11,29). The consistent finding of incomplete monozygotic concordance in twin studies of both autism and ADHD (11,30) as well as data demonstrating that even in genetic syndromes highly associated with ASD, a significant percentage of carriers do not express autistic phenotypes (11,25), are consistent with a model in which environmental factors interact with genetic susceptibilities to influence ASD/ADHD risk, clinical phenotype and/or treatment outcome (11,29). Given the extensive literature documenting chemical-induced mutagenesis, the identification of *de novo* gene mutations associated with clinical diagnosis of ASD (11,22,25) is also consistent with the idea of environmental risk modifiers for ASD and other neurodevelopmental disorders.

More definitive human evidence corroborating a role for environmental factors has recently been reported. In one of the largest twin studies conducted to date, 192 mono- and dizygotic twin pairs were analyzed to quantify the relative

contributions of genetic heritability versus the shared environment. The findings from these analyses suggested that 38% of ASD cases are attributable to genetic causes whereas 58% are linked to the shared *in utero* environment (12). The model used in this study had a number of inherent biases (e.g., it was assumed there were no gene x environment interactions, monozygotic and dizygotic twins were assumed to share the environment to the same extent, and there are questions regarding the validity of the values used for the prevalence for autism and ASD). However, similar conclusions were recently reached in an independent study of over 14,000 children with autism in Sweden that demonstrated a heritability of 50%, supporting an equally strong role for environmental risk factors (31). Collectively, these studies suggest that environmental factors can significantly influence susceptibility and the variable expression of traits related to neurodevelopmental disorders, thereby providing a plausible explanation for both the dramatic increase in the prevalence of complex neurodevelopmental disorders and the significant clinical heterogeneity that is a hallmark of both autism and ADHD (11,32).

Environmental factors associated with increased risk for neurodevelopmental disorders

Early indications of an environmental contribution to neurodevelopmental disorders came from observations of a high incidence of autism associated with congenital rubella (33). Subsequent studies linked prenatal infections to increased risk for not only ASD but also other neurodevelopmental disorders, particularly schizophrenia (34-37), and expanded the range of non-genetic risk factors for neurodevelopmental disorders to include other intrauterine stresses (38-42), paternal age (42-45) [but see (46)], maternal nutrition and metabolic status (11,47-49), and hormones, including sex hormones that contribute to the well-documented

sex differences in autism susceptibility (50) *via* gene-specific epigenetic modifications of DNA and histones (51).

Early reports that *in utero* exposure to valproic acid or thalidomide during critical periods of development was associated with increased expression of autism-related traits in children (52) raised significant interest regarding the role of chemical exposures in neurodevelopmental disorders. Subsequent epidemiological studies reported an increased risk for ASD and other neurodevelopmental disorders associated with maternal use of various medications and drugs of abuse, including alcohol (52-58), as well as prenatal or early postnatal exposure to diverse environmental chemicals. Environmental chemicals postulated to confer risk for neurodevelopmental disorders include legacy chemicals known to be toxic to the developing human nervous system, such as lead, mercury and polychlorinated biphenyls (PCBs) (2,59,60), as well as more contemporary contaminants such as pesticides, including organophosphorus (OP) and organochlorine (OC) pesticides, neonicotinoids and pyrethroids, flame retardants including the polybrominated diphenyl ethers (PBDEs), plasticizers such as phthalates and bisphenol A (BPA), and complex environmental mixtures such as air pollution and cigarette smoke (11,14,32,61-63).

The epidemiological data linking environmental chemicals to increased risk for ADHD (32) or ASD (62) were recently critically reviewed, and the major conclusions from these reviews are briefly summarized here. Polanska and colleagues (32) reviewed epidemiologic studies of environmental chemicals and ADHD published in peer-reviewed English language journals since the year 2000. Out of a total of 72 articles identified in their initial literature search, 40 met their inclusion criteria. Many of these studies focused on exposure to tobacco smoke,

and were largely consistent in identifying a positive association between tobacco smoke exposure and a diagnosis of ADHD or expression of ADHD symptoms in children (32). While the strength of the association differed between studies, the authors concluded that it generally appeared that the children of smokers are approximately 1.5-3 times more likely to have ADHD or ADHD symptoms than the children of non-smokers. Other environmental chemicals addressed in the articles included in their review were ethanol, phthalates, BPA, polyfluoroalkyl chemicals (PFCs) and polycyclic aromatic hydrocarbons (PAHs). Interestingly, while a number of studies in experimental animal and cell-based models suggest that developmental exposure to these chemicals elicits outcomes with face validity to at least a subset of ADHD-like symptoms [reviewed in (32)], the authors found no consistent findings in the epidemiological literature of an association between early life exposure to any of these environmental chemicals and increased risk for ADHD (32). However, the authors concluded that there were an insufficient number of studies of the impact of phthalates, BPA, PFCs, PAHs and alcohol on ADHD to allow for meaningful conclusions as to whether these chemicals may indeed be environmental modifiers of ADHD risk.

Polanska and colleagues did not comment on the literature implicating lead and PCBs as risk factors for ADHD although a recent review by Eubig, Aguiar and Schantz (64) present a compelling case for such associations. The analysis by Eubig and colleagues demonstrated that studies of lead exposure in children provide evidence for impairments in executive function and attention that parallel those observed in children diagnosed with ADHD. Animal studies of developmental lead exposure indicate behavioral and neurochemical changes with face validity to ADHD (64). Importantly, an association between blood lead levels and a diagnosis of

ADHD has been reported in a number epidemiologic studies [reviewed in (64)]. Similarly, the epidemiologic evidence of PCB effects on children shows parallels to behavioral domains affected in ADHD, although these were subtly different from the deficits noted in lead-exposed children (64). The findings from animal studies of developmental PCB exposures reveal deficits on behavioral tasks that assess many of the same cognitive domains that are impaired in children with ADHD, as well as many changes in dopamine neurotransmission that are reflective of neurochemical changes associated with ADHD (64). However, the relationship between PCB exposure and ADHD in children remains largely unexplored.

The epidemiologic evidence linking environmental chemical exposures and ASD was also very recently reviewed by Kalkbrenner and colleagues (62). These authors identified 58 relevant articles in the peer-reviewed literature published prior to March 1, 2014, of which 32 met their inclusion criteria (individual-level data on autism, exposure measures pertaining to pregnancy or the 1st year of life, valid comparison groups, control for confounders and adequate sample sizes). These 32 articles included studies of autism and estimates of exposure to tobacco, air pollutants, volatile organic compounds and solvents, metals, pesticides, PCBs, PBDEs, PFCs, BPA and phthalates. As with the studies of these chemicals in relation to ADHD risk, many of these chemicals have been shown to cause effects in experimental animal or cell-based models that have face validity to behavioral outcomes or cellular phenotypes associated with autism [reviewed in (62,65)]. Some of these environmental exposures also showed associations with autism in the analysis of the epidemiologic literature by Kalkbrenner and colleagues. The most strongly and consistently associated were traffic-related air pollutants, some metals and the OP and OC pesticides, with suggestive trends for some volatile

organic compounds and phthalates (62). Interestingly, neither tobacco smoke, which was strongly associated with increased ADHD risk (32), nor alcohol showed a consistent association with increased risk for ASD.

All three of these comprehensive analyses of the human literature reached similar broad conclusions: the relevant publications that are currently available, with the possible exception of studies of tobacco and alcohol, are too limited in scope to either infer causality or to rule out the possibility that these or additional environmental chemicals confer risk for ADHD or ASD (32,62,64). All three sets of authors identified similar challenges facing epidemiologic studies of environmental chemicals and neurodevelopmental disorders. These include difficulties inherent in most epidemiologic studies, such as obtaining accurate measures of exposure, particularly for chemicals with short half-lives such as some of the pesticides, phthalates and BPA, controlling for confounding factors, especially socioeconomic stressors that tend to co-vary with environmental exposures, and dealing with co-exposures, a not insignificant issue in light of reports that 250 xenobiotic chemicals were detected in biological samples from a 2013 representative sample of the U.S. in the National Health and Nutrition Examination Survey. However, epidemiologic studies of environmental factors in complex neurodevelopmental disorders face an additional challenge in that these disorders are phenotypically heterogeneous. Children are diagnosed as having ASD or ADHD based on observable symptom clustering, not causal pathways, a phenomenon known as etiological heterogeneity (66-68). The complexity of heritable risk factors contributing to neurodevelopmental disorders like ADHD and ASD likely creates a range of sensitivities to environmental risk factors (29), which masks clear associations between exposure and diagnosis (see Figure 1). It has been proposed that a more

nuanced “phenome” approach that identify populations based on distinctive phenotypes may aid in the further discovery of modifiable environmental chemical risk factors that impact neurodevelopment until such time as mechanism-based biomarkers for distinctive and discrete etiologies are available (62).

[Place Figure 1 approximately here]

Mechanisms by which environmental factors influence risk of neurodevelopmental disorders

Genetic, histologic, *in vivo* imaging and functional data are converging on altered patterns of neuronal connectivity as the biological basis underlying the behavioral and cognitive abnormalities associated with many neurodevelopmental disorders and intellectual disabilities (69-74). The candidate genes most strongly implicated in the causation of neurodevelopmental disorders encode proteins that regulate the patterning of neuronal networks during development and influence the balance of excitatory to inhibitory synapses (65,69,75,76), Histological studies report increased spine density in Golgi-impregnated pyramidal neurons of Layer II in the frontal, temporal and parietal lobes and Layer V of the temporal lobe in ASD patients (77), suggesting skewed cortical synaptic activity towards excitation, although functional evidence of excitation is needed to further support this hypothesis. Abnormal spine morphology is also found in the temporal and visual cortices of patients with Fragile-X, a syndrome with high incidence of ASD (78) and in patients with schizophrenia (79,80). Neuroimaging studies of children with ASD suggest a pattern of early brain overgrowth in the first few years of life followed by

aberrant maturation in adolescence while functional analyses suggest impaired long-range connectivity but increased local and/or subcortical connectivity [reviewed in (81)]. In contrast, in childhood onset schizophrenia, deficits in cerebral volume, cortical thickness and white matter maturation seem most pronounced in childhood and adolescence and may level off in adulthood, and functional analyses suggest deficits in local connectivity, with increased long-range connectivity [reviewed in (81)]. Thus, even though ASD and childhood onset schizophrenia are thought to share an underlying genetic architecture (73,82-84), the dynamic changes that occur in the developing brain of children diagnosed with these neurodevelopmental disorders vary across space and time. These differences perhaps reflect the influence of differential environmental modifiers acting on a shared genetic substrate. Alternatively, these differences may reflect differences in the timing of exposure, a factor well-known to influence neurodevelopmental outcomes following exposure to ethanol (85).

It has been proposed that these inherent imbalances in synaptic connectivity in children at risk for neurodevelopmental disorders likely provide the biological substrate for enhanced susceptibility to environmental triggers that modulate neurodevelopmental processes that determine synaptic connectivity, including neuronal migration, apoptosis, elaboration of axons and dendrites, and activity-dependent refinements of synaptic connections (65,76). Perturbations of the spatiotemporal patterns or magnitude of any of these events can interfere with the formation of functionally meaningful networks and give rise to the deficits in functional connectivity associated with neurodevelopmental disorders (76,86). Research findings from studies of transgenic animal models expressing neurodevelopmental disorder risk genes further support the hypothesis that the

changes in synaptic connectivity observed in children at risk for neurodevelopmental disorders arise from perturbations of dendritic growth, synapse formation and synapse stabilization (69,82).

Diverse pathophysiologic mechanisms have been hypothesized to mediate interactions between environmental chemicals and genetic risk factors that influence neuronal connectivity. These include: a) mutations in genes that encode proteins involved in detoxification of endogenous toxins and xenobiotics, such as the enzyme paraoxonase 1 (PON1), which hydrolyzes OP pesticides and has been associated with increased risk of autism [reviewed in (11)]; b) endocrine disruption, based on the rationale that ASD and ADHD disproportionately affect males (87) and the requirement for thyroid hormone and other hormones for normal neurodevelopment (88,89); c) chemical perturbation of the microbiome, derived from evidence suggesting that microbiota may regulate responses to xenobiotics and influence brain function, and that the microbiota may differ in children with neurodevelopmental disorders versus typically developing children (90,91); and d) mitochondrial dysfunction and oxidative stress (11,92-94). While there is experimental evidence demonstrating that environmental chemicals can disrupt endocrine signaling, alter the microbiome and cause oxidative stress, and neurodevelopmental disorders have been associated with endocrine disruption, altered gut microbiota and biomarkers of oxidative stress in the central nervous system, causal relationships between the chemical effect on these endpoints and increased risk of neurodevelopmental disorders are largely lacking. Additional hypotheses involving immune dysregulation, epigenetics and the convergence of environmental factors and genetic susceptibilities on neurotransmitters or signaling

pathways that critically influence neuronal connectivity are discussed in more detail in the following paragraphs.

[Place Table 1 approximately here]

Epigenetic mechanisms. Perhaps the most theoretically straightforward mechanisms of interaction between environmental factors and susceptibility genes are physical interactions between chemicals and DNA (e.g., chemically-induced *de novo* mutations) or chromatin (e.g., epigenetic changes in miRNA expression, DNA methylation patterns and/or histone acetylation) (95,96). While experimental and clinical studies clearly demonstrate that epigenetic mechanisms are critically involved in regulating normal neurodevelopment, synaptic plasticity and cognitive function, and that epigenetic changes in the brain are associated with neurodevelopmental disorders (97-101), there is as yet little data causally linking environmental chemicals to neurodevelopmental disorders via epigenetic mechanisms. One of the few potentially relevant examples is the recent demonstration that the non-dioxin-like (NDL) PCB congener, PCB 95, increased synaptogenesis via upregulated expression of miR132 (102). miR132 represses expression of the transcriptional repressor, methyl-CpG-binding protein-2 (MeCP2) (103). MeCP2 dysfunction is linked to dendritic and synaptic dysregulation (104), and mutations in MeCP2 are the genetic cause of Rett syndrome and are associated with increased ASD risk (105-107). miR132 also interacts with fragile X mental retardation protein (FMRP) to regulate synapse formation (108), and more recently, expression of miR132 was shown to be dysregulated in schizophrenia (109,110). Intriguingly, a recent analysis of persistent organic pollutants in children's brains

found significantly higher levels of PCB 95, the same congener found to influence miR132 levels, in postmortem brains of children with a syndromic form of autism, as compared to neurotypical controls (111). In contrast, levels of other PCBs and PBDEs were comparable across groups. The samples with detectable PCB 95 levels were almost exclusively those with maternal 15q11-q13 duplication (Dup15q) or deletion in Prader-Willi syndrome. When sorted by birth year, Dup15q samples represented five of six samples with detectable PCB 95 levels and a known genetic cause of ASD born after the 1976 ban on PCB production. Dup15q was the strongest predictor of PCB 95 exposure across age, gender, or year of birth. Dup15q brain samples had lower levels of repetitive DNA methylation as measured by LINE-1 pyrosequencing, although methylation levels were confounded by year of birth. Collectively, these results suggest the possibility that NDL PCBs contribute to an increased risk of neurodevelopmental disorders via epigenetic mechanisms. This is of potential significance since recent studies clearly demonstrate that NDL PCBs remain a current and significant risk to the developing human brain. First, contemporary unintentional sources of NDL PCBs have been identified, most notably commercial paint pigments (112). Second, in the past year it was reported that PCB levels in the indoor air of elementary schools in the United States exceed the EPA's 2009 public health guidelines, and the NDL congener PCB 95 was the second most abundant congener identified in indoor school environments (113). Third, the latest NHANES study confirmed widespread exposure to PCBs among women of childbearing age living in the United States (114).

Immune dysregulation. Crosstalk between the nervous and immune systems is essential for normal neurodevelopment (115-117). Multiple cytokines are normally produced in the healthy brain where they play critical roles in most

aspects of neurodevelopment, including neuronal differentiation, synapse formation and structural plasticity (117,118). Two cytokine classes of particular interest in neurodevelopmental disorders include the neuropoietic cytokines, exemplified by IL-6 (115,119), and the type II interferon, gamma interferon (IFNɣ) (120). The neuropoietic cytokines interact directly with diverse neuronal cell types *in vitro* to modulate dendritic growth and synapse formation (121-123), and *in vivo* to regulate synapse elimination in the developing rodent brain (124). Chronic IL-6 overexpression reduces neuronal expression of glutamate receptors and L-type Ca^{2+} channels *in vitro* and *in vivo (125)*. IFNɣ also modulates dendritic growth via effects on STAT1 (126) and MAPK signaling (127). Increased IFNɣ skews the balance of excitatory to inhibitory synapses towards increased excitatory signaling in cultured hippocampal neurons (128); whereas decreased IFNɣ triggers decreased density of pre-synaptic terminals in the spinal cord of adult mice (129). Based on these observations, it is perhaps not surprising that a growing body of evidence supports a key role for immune system dysregulation in neurodevelopmental disorders (120,130-133). As discussed above, maternal infection during pregnancy is associated with increased risk of neurodevelopmental disorders (115), and experimental evidence that many cytokines can cross the placenta and blood-brain barrier (119). Collectively, such studies illustrate the potential for cytokines altered by maternal infection to adversely impact neurodevelopment during gestation. Whether immune and nervous system dysfunction in neurodevelopmental disorders reflect parallel outcomes or whether deficits in one system contribute to deficits in the other remain open to speculation (115). Another unanswered question is whether environmental chemicals known to influence immune status can cause

adverse neurodevelopmental outcomes of relevance to ASD and other disorders by altering cytokine profiles in the developing brain.

Convergence of environmental factors and genes on critical signaling pathways. One fundamental way in which heritable genetic vulnerabilities might amplify the adverse effects triggered by environmental exposures is if both factors (genes *x* environment) converge to dysregulate the same neurotransmitter and/or signaling systems at critical times during development (29,65). Evidence from both human genetics studies and experimental models expressing genetic risk factors has yielded the startling fact that many ASD risk genes converge on several major signaling pathways that play key roles in regulating neuronal connectivity in the developing brain (25,69,82,134,135). Data emerging from studies of syndromic ASD and rare highly penetrant mutations or CNVs in ASD have identified distinct adhesion proteins needed to maintain and modify synapses in response to experience, as well as intracellular signaling pathways that control dendritic arborization and/or synaptogenesis that appear to represent convergent molecular mechanisms in ASD. The former includes neuroligins, neurexins, contactin-associated protein-2 (CNTNAP2) and SHANK proteins (24,26,69,136); the latter, ERK and PI3K signaling (25), as well as Ca^{2+}-dependent signaling (134). Many of these same signaling pathways have also been implicated in schizophrenia risk (73,82-84).

Dendritic arborization and synaptic connectivity are determined by the interplay between genetic and environmental factors (137-139). During normal brain development, neuronal activity is a predominant environmental factor in shaping dendritic arbors and synaptic connectivity. The importance of neuronal activity is evidenced by the remarkable effect of experience on the development

and refinement of synaptic connections, which not only patterns neural circuitry during development (140,141) but also underlies associative learning throughout life (142). The dynamic structural remodeling of dendrites that occurs during development is driven in large part by Ca^{2+}-dependent signaling pathways triggered by NMDA receptor activation and other extrinsic cues such as hormones and neurotrophins (143,144). NMDA receptor-mediated Ca^{2+}-dependent signaling couples neuronal activity to dendritic arborization, and this is mediated by sequential activation of CaMKK, CaMKI and MEK/ERK to enhance CREB-mediated transcription of Wnt2 (145). A number of environmental chemicals modulate calcium signaling (13), but thus far, only NDL polychlorinated biphenyls (PCBs) that bind to the ryanodine receptor (RyR) have been found to trigger this same Ca^{2+}-dependent signaling pathway to enhance dendritic growth in primary cultures of rat hippocampal neurons (146). These effects of PCB 95 map onto signaling pathways implicated in neurodevelopmental disorders. For example, Timothy syndrome, which is caused by a gain-of-function mutation in the L-type Ca^{2+} channel CaV1.2, has a 100% incidence of neurodevelopmental disorders and 60% rate of autism (147). Neurons differentiated from induced pluripotent stem cells derived from Timothy syndrome patients exhibit increased Ca^{2+} oscillations and upregulated expression of genes linked to Ca^{2+}-dependent regulation of CREB, including CaMK (148). Collectively, these studies support the hypothesis that NDL PCBs amplify the risk and/or severity of NDD by converging on signaling pathways targeted by heritable defects in Ca^{2+}-dependent signaling pathways that regulate neuronal connectivity.

[Place Figure 2 approximately here]

The hypothesis of an imbalance between excitation and inhibition within developing CNS circuits as a common etiological factor contributing to autism, fragile X and schizophrenia is supported by several reports of GABA receptor abnormalities in these neurodevelopmental disorders (149). A significantly lower level of $GABR\beta_3$ expression has been documented in postmortem brain samples obtained from children diagnosed with autism, Rett Syndrome and Angelman Syndrome (150). The gene encoding for $GABR\beta_3$ is located in a non-imprinting region of chromosome 15q11-13 suggesting an overlapping pathway of gene dysregulation common to all three disorders that may stem from abnormal regulation of DNA methylation by the methyl CpG binding protein MeCP2 (151). Quantitative immunoblot analyses of the brains of Mecp2 deficient mice, a model of human Rett syndrome, are deficient in the $GABR\beta_3$ subunit (150), further supporting the working hypothesis that epigenetic mechanisms conferring gene dysregulation within 15q11-q13 in Angelman syndrome and autism may significantly contribute to heightened susceptibility to compounds that potently block the chloride channel of $GABR\beta_3$. Autism has been linked to polymorphisms within additional genes that encode GABR, including $GABR\gamma_1$ located on 15q11-13 (152,153). Complex epistatic interactions between genes that encode $GABR\alpha_4$ and $GABR\beta_1$ within 4q12 have also been reported in autistic probands (154).

Collectively, these data implicate GABAR dysregulation as a major contributor to imbalances between excitation and inhibition in children whose brains may be vulnerable to environmental modulation of epigenetic mechanisms and/or GABA receptor expression and/or function. Relevant to the latter are the organochlorine (OC) pesticides that possess polychloroalkane structures and are known to bind to

GABR in the mammalian brain, many with nanomolar affinity, to potently block Cl^- conductance through the receptor (14). In the United States, toxaphene represents one of the most heavily used OC pesticides but it was banned in 1990; the OC pesticides heptachlor and dieldrin were banned in the late 1980s. Examples of more recently used polychloroalkane insecticides include endosulfan and lindane. Dicofol, an OC structure similar to DDT, is currently registered for use on agricultural crops. Because of their chemical stability, global distribution from countries that continue to use these compounds, and their propensity to bioaccumulate, exposures to OC insecticides continue to be a significant concern to human health worldwide. Another class of insecticide that interferes with GABA neurotransmission and has attained broad domestic and commercial use is the 4-alkyl-1-phenylpyrazoles. Although these compounds do not persist in the environment to the same extent as OC pesticides, they are heavily used within the home and for commercial pest control. For example, fipronil is formulated as a topical for control of fleas and ticks on pets. Fipronil's insecticidal activity is mediated primarily through its actions as a non-competitive antagonist of GABR. In this regard, fipronil was initially developed as an insecticide because of its higher selectivity for insect versus mammalian GABR (155). However, results from recent studies indicate that fipronil, like endosulfan and lindane, is indeed a high affinity noncompetitive antagonist for the mammalian β_3-homopentameric GABR (156,157). In fact, fipronil binds the GABRβ_3 with nanomolar affinity in a manner indistinguishable from its interaction with insect GABA receptors. These findings raise questions about the high degree of selectivity originally attributed to 4-alkyl-1-phenylpyrazoles. More importantly, the finding that a diverse group of widely used insecticides converges on a common molecular target – the mammalian GABRβ3 –

has important implications for human risk, especially in those individuals with heritable impairments in GABA receptor signaling pathways.

Conclusions

A significant contribution from environmental factors in determining the risk for neurodevelopmental disorders is consistent with both the rapid increase in prevalence of these disorders and the clinical heterogeneity that is the hallmark of the two most common complex disorders, ASD and ADHD. However, this phenotypic heterogeneity together with the complex multigenic etiologies of these disorders significantly increases the difficulty of identifying specific environmental factors that confer increased risk. If the complexity of heritable factors contributing to susceptibility to neurodevelopmental disorders creates a range of sensitivities of the developing brain to the adverse effects of environmental factors (11,25,29), then establishing clear associations between exposure to environmental factors and diagnosis of specific neurodevelopmental disorders will be challenging.

Several signaling pathways and developmental processes have emerged from genetic studies as convergent molecular mechanisms or targets in neurodevelopmental disorders, and data is already accumulating of environmental chemicals that potentially interact with those mechanisms or targets. These emerging data suggest a biological framework for not only studying mechanisms by which specific environmental and genetic factors interact to confer risk for neurodevelopmental orders but also for informing epidemiological studies and for setting up *in vitro* and simple model systems to screen environmental chemicals for their potential to increase risk of neurodevelopmental disorders.

Clearly, work is urgently needed to better predict which combination of defective genes and environmental exposures pose the greatest risk for neurodevelopmental disorders. The fact that chemical exposures are more readily controlled than genetic factors to prevent or mitigate the expression of phenotypic traits related to neurodevelopmental disorders coupled with the significant toll that neurodevelopmental disorders and disabilities exact on the affected individual, their families and society, provide compelling reasons to engage in this endeavor.

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Table 1. Mechanisms hypothesized to mediate gene-environment interactions that influence the risk and/or severity of neurodevelopmental disorders (NDD).

Mechanism	Rationale	Key references
Heritable deficits in xenobiotic metabolism	Decreased ability to detoxify environmental chemicals might effectively increase the neurotoxic potential of an environmental chemical	11
Endocrine disruption	Many NDDs are predominant in one sex and many hormones are required for normal neurodevelopment (sex steroids and thyroid hormones) or have significant effects on neurodevelopment (glucocorticoids).	87, 88, 89
Disruption of the gut microbiome	Emerging evidence indicates that the gut microbiome regulates host response to pathogenic microbial or xenobiotic exposures, and the gut microbiota in children with autism differs from that of neurotypical children.	11, 92, 93, 94
Epigenetic	Environmental chemicals have been demonstrated to alter DNA methylation, histone acetylation and miRNA expression profiles, and these parameters are altered in at least some children with NDDs	95 – 102, 111
Immune dysregulation	Crosstalk between the nervous and immune systems is essential for normal neurodevelopment, environmental chemicals can alter immune function, and there is increasing evidence of immune dysregulation in NDDs	115-117, 130-133
Convergence of environmental and genetic factors on signaling pathways	Many genetic risk factors for NDDs converge on several major signaling pathways that critically regulate synaptic connectivity in the developing brain; and environmental chemicals have been identified that target these same signaling pathways.	13, 29, 65

Figure legends

Figure 1. The challenge of using epidemiological approaches to identify environmental risk factors for neurodevelopmental disorders (NDD). In any population, including the neurotypical population, variation in the genetic make-up, timing of exposure, nutritional status and/or coincidental illnesses will create a range of vulnerabilities to the developmental neurotoxicity of an environmental chemical. The TD_{50} is the exposure dose of the environmental chemical (or chemical mixture) that produces a phenotypic trait of relevance to a neurodevelopmental disorder. The genetic heterogeneity associated with risk for neurodevelopmental disorders like ADHD and ASD likely creates a range of sensitivities to environmental risk factors; however, stratification of neurodevelopmental disorders based on distinctive phenotypes may result in definable subpopulations with different sensitivity to environmental modifiers of NDD-related deficits, severity and/or treatment outcomes.

Figure 2. Interactions between genetic susceptibilities and environmental factors likely determine the risk and/or phenotypic expression of many neurodevelopmental disorders. If the environmental factor is a chemical, then the timing of exposure relative to the stage of neurodevelopment may influence the impact of the environmental chemical. The mechanism(s) by which environmental chemicals modify genetic risks to influence neurodevelopmental outcome are largely unknown. One possibility illustrated in this figure is that the genetic factor (heritable defects in Ca^{2+} signaling) and environmental factor (non-dioxin-like PCBs) converge on the same critical signaling pathway (Ca^{2+}-dependent activation of the CaMK-CREB-Wnt signaling pathway) to amplify adverse effects on a

neurodevelopmental endpoint (synaptic connectivity) of relevance to neurodevelopmental disorders.