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Immunologic and Neurodevelopmental Susceptibilities of Autism

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Abstract

Symposium 5 focused on research approaches that are aimed at understanding common patterns of immunological and neurological dysfunction contributing to neurodevelopmental disorders such as autism and ADHD. The session focused on genetic, epigenetic, and environmental factors that might act in concert to influence autism risk, severity and co-morbidities, and immunological and neurobiological targets as etiologic contributors. The immune system of children at risk of autism may be therefore especially susceptible to psychological stressors, exposure to chemical triggers, and infectious agents. Identifying early biomarkers of risk provides tangible approaches toward designing studies in animals and humans that yield a better understanding of environmental risk factors, and can help identify rational intervention strategies to mitigate these risks.

I. How Can Chemical Exposure Contribute to Autism Risk? I. N. Pessah, UC Davis, CA, USA

Autism is a heterogeneous neurodevelopmental disorder defined by core deficits in social reciprocity, communication, and restrictive/repetitive patterns of interest and behavior (American Psychiatric Assoc, 2000). Although autism may be one of the most heritable complex disorders, the genes linked to autism risk do not segregate in a simple Mendelian manner (Folstein *et al.*, 2001; Trikalinos *et al.*, 2005). There is increasing scientific interest in identifying complex interactions among multiple genes that contribute to autism risk, and non-heritable environmental exposures may significantly influence susceptibility and variable expression of autism and autism-related traits. A major challenge in the field is to identify environmental factors of relevance to autism. From a toxicologist's perspective, the identity of "defective" genes and signaling pathways linked to autism provide important clues about exposures to environmental chemicals that influence autism susceptibility, severity, and/or treatment outcomes. One fundamental way by which heritable genetic vulnerabilities can amplify the adverse effects triggered by environmental exposures is if both factors (genes × environment) converge to dysregulate the same neurotransmitter and/or signaling systems at critical times of development. Current research is being directed at defining distinct

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neurological endophenotypes in autism that may possess overlapping sets of susceptibility genes (Muller, 2007). It is therefore reasonable to expect that as subgroups of autistic children are better defined based on common biological abnormalities, distinct sensitivity to environmental modifiers of autism risk, severity and/or treatment outcomes will be identified. Current efforts to identify clinical endophenotypes within the autism spectrum are therefore likely to help our understanding of the constellations of genes that confer differential sensitivity to distinct environmental exposures during gestational and neonatal development. Such approaches will likely prove useful in defining subgroups of children that differ in susceptibility to environmental exposures that promote autism risk and severity.

Understanding how low-level chemical exposure influences molecular, cellular and behavioral outcomes relevant to autism will better inform geneticists, neuroscientists and immunologists about autism's complex etiologies. Recent genetic findings coupled with emerging histological, electrophysiological and functional imaging studies suggest that autism results from an imbalance in the ratio of excitatory and inhibitory neurons within the developing brain (Rubenstein and Merzenich, 2003) and the neural networks they form (Belmonte and Bourgeron, 2006). Such imbalances are likely to result in the failure of connections to form normally between brain regions involved in higher-order associations (Geschwind and Levitt, 2007). The inherent imbalances in neuronal connectivity present in children at risk for autism are likely to provide the biological substrate for enhanced susceptibility to environmental triggers that are known to target signaling systems that establish the basic patterns of connectivity, from early neuronal migration and axonal pathfinding to disruption of later postnatal events that function to refine neuronal connections, such as dendritic growth, synapse formation and pruning (Belmonte and Bourgeron, 2006; Geschwind and Levitt, 2007; Diccio-Bloom, 2006). The toxicological literature includes ample evidence that many of the environmental chemicals of concern to human health either directly or indirectly affect the signaling systems that are impaired in autism. For example, several pesticides of historical and current importance are in fact known to interfere with acetylcholine (ACh) and γ -aminobutyric acid (GABA) neurotransmission, which are neurotransmitter systems altered in subsets of autistic individuals. Another example is the possible role of persistent organic pollutants that directly alter Ca^{2+} signaling pathways and Ca^{2+} -dependent effectors and are thus likely to impact a broad range of transmitter systems, including cholinergic, GABAergic, and dopaminergic.

Chemicals that interfere with cholinergic neurotransmission

Organophosphates (OPs) are potent inhibitors of the enzyme acetylcholinesterase (AChE) that is responsible for rapid hydrolysis of acetylcholine at nicotinic and muscarinic synapses. Inhibition of AChE by OPs has been amply documented as causing a plethora of toxicological effects that result from initially overstimulating, and consequently desensitizing, cholinergic transmission. More recent research suggests that some OPs cause developmental neurotoxicity by mechanisms independent of AChE inhibition (Pope, 1999; Schuh *et al.*, 2002; Jameson *et al.* 2007; Ricceri, *et al.*, 2006; Slotkin *et al.*, 2007). For example, the OP chlorpyrifos has been shown to enhance the phosphorylation of the Ca^{2+} /cAMP response element binding protein CREB in neuronal cultures at concentrations well below those that inhibit AChE (Schuh *et al.*, 2002). Tight regulation of CREB activity by Ca^{2+} -dependent phosphorylation is critical for normal neural progenitor proliferation and differentiation, dendritic development and cognitive function (Peltier *et al.*, 2007; Redmond and Gosh, 2005). CREB activity in turn regulates the expression of many nerve-specific genes including NF-1, TH, chromogranin, pEJ, and a number of immediate early genes that encode transcription factors (e.g., c-Jun). Slotkin and co-workers recently showed that neonatal exposure of rats to two widely used OPs, chlorpyrifos and diazinon, produced marked changes in the pattern of expression of specific isoforms of fibroblast growth factor genes (*fgf*) and their receptor (*fgfr1*) at doses below those

that cause systemic toxicity or growth impairment resulting from AChE inhibition (Slotkin *et al.*, 2007). Although the molecular mechanisms mediating these developmental effects are unknown, they may have a common origin since the nuclear accumulation of FGFR1 protein is activated by changes in cell contacts and by stimulation of cells with growth factors, neurotransmitters and hormones. Stachowiak and co-workers proposed that FGFR1 represents a key convergence point for integrating signaling cascades initiated by specific membrane receptors that transmit signals to sequence specific transcription factors through co-activation of CREB (Stachowiak *et al.*, 2003). Investigation into the adverse toxicological consequences of OPs *via* AChE-dependent and independent mechanisms and how they can be synergized by inborn errors in Ca²⁺ signaling such as Timothy Syndrome (see below), which has a 60% autism rate, are needed to permit more meaningful risk assessments of susceptible populations.

Neurotransmission at cholinergic synapses is essential for functional aspects of cognition, reward, motor activity and analgesia. In addition, dysregulation of cholinergic neurotransmission has been associated with several pathological conditions including autism. Regional differences in the expression of specific nAChR subunits have been documented in postmortem adult brains obtained from autistic and non-autistic individuals. A decrease in the density of [³H]epibatidine binding sites within the parietal cortex, cerebellum and thalamus of autistic brains was correlated to decreases in the levels of $\alpha 3$, $\alpha 4$ and $\beta 2$ subunits by western blotting (Martin-Ruiz *et al.*, 2004; Lee *et al.*, 2002; Perry *et al.*, 2001). In the cerebellum, the [³H]epibatidine binding density to $\alpha 3$ and $\alpha 4$ subunits was reduced by 40–50% in the granule cell, Purkinje and molecular layers in the autistic group compared with the non-autistic group. Cerebella from autistic patients possessed approximately 3-fold higher binding of [¹²⁵I] α -bungarotoxin to the $\alpha 7$ subunit in the granule cell layer compared to the general population and developmental delay comparison groups (Perry *et al.*, 2001). How these differences in nAChR expression arise is unclear. However, these findings raise important questions as to whether deficits in nAChR neurotransmission identified in at least a subset of autistic children might confer a particularly high sensitivity to the adverse effects of OPs and/or neonicotinoid exposure. Allelic variants of the gene that encodes for paraoxonase (PON), a metabolic enzyme that is critical to the detoxification of OPs, have been associated with autism in North America and these variants appear to have lower metabolic activity *in vitro* (D'Amelio *et al.*, 2005). Lower PON activity was also recently associated with autism in a Romanian study (Pasca *et al.*, 2007). Thus, reduced metabolic capacity to detoxify and excrete OPs, in conjunction with deficits in nAChR neurotransmission could provide a framework for future studies of gene \times environment interactions relevant to autism risk. Environmental epidemiological studies of possible associations between pesticide exposures and the major triad of deficits seen in autism (social reciprocity, language, and stereotyped behavior) have only begun to receive attention (Hertz-Picciotto *et al.*, 2006). Eskenazi and co-workers recently reported evidence of a positive association between levels of dialkylphosphate metabolites (biomarkers of OP exposure) in maternal and child urine with risk of pervasive developmental disorder at 24 months of age in a longitudinal birth cohort of primarily Latino farm worker families in California (Eskenazi, *et al.*, 2007). Although these new associations between OP exposure and pervasive developmental disorders should not be interpreted as evidence of causation, clearly more research is needed to understand how long-term low level exposure to OPs, neonicotinoids and other chemicals that interfere with cholinergic signaling influence adverse developmental outcomes, especially in children in whom these signaling systems are genetically impaired.

Chemicals that interfere with GABA neurotransmission

Several classes of pesticides are known to interfere with GABA-mediated neurotransmission because they bind to the type A family of GABA receptors (GABR) and block their ability to mediate chloride fluxes. Along with nAChRs discussed above, GABR channels are members of the ligand-gated ion channel superfamily of genes that code for proteins having high

sequence homology, but divergent spatiotemporal expression patterns and functions. In immature neurons, GABR primarily mediate excitatory responses, but during maturation and in mature neurons they mediate rapid inhibition of transmission (Michels *et al.*, 2007). This “switch” in function is the result of a reversal in direction of the chloride gradient across the neuronal membrane with the maturation-dependent expression of the potassium-chloride co-transporter KCC2 that lowers the internal Cl concentration below that found in the extracellular fluid (Lu *et al.*, 1999). The physiological relevance of these different functional GABR responses during neuronal maturation remains unclear. What is clear is that altered expression and function of these chloride channels have been implicated in autism and associated comorbidities including sleep disturbances, anxiety, and epilepsy.

Organochlorine (OC) insecticides were widely used for both large-scale agriculture and domestic pest control beginning in the early 1940s. The vast majority of OC insecticides were banned from use in the United States beginning in the late seventies due to their persistence in the environment, adverse effects on avian reproduction, and their ability to promote hepatic tumors in rodent studies. OC insecticides that possess polychloroalkane structures are known to bind to GABR in the mammalian brain and potently block their ability to conduct Cl⁻, with many having nanomolar affinity for their receptor binding site (Cole *et al.*, 1986; Lawrence and Casida, 1984). In the United States, toxaphene represents one of the most heavily used pesticides and was banned in 1990, whereas heptachlor and dieldrin were banned in the late 1980s. Examples of polychloroalkane insecticides that are currently being used in the United States include endosulfan and lindane. Dicofol, an OC structure similar to DDT, is also 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. Yet relatively little is known about their developmental neurotoxicity, and the long-term consequences of low-dose exposures have not been adequately evaluated (Slotkin *et al.*, 2007).

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 extent observed with OCs, 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 forms of GABR relative to mammalian GABR (Bloomquist 2003). 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 (GABR β_3) (Chen *et al.*, 2006; Sammelson *et al.*, 2004). In fact, the GABR β_3 recognizes fipronil with nanomolar affinity in a manner indistinguishable from its interaction with insect GABA receptors. These new findings raise questions about the high degree of selectivity once attributed to 4-alkyl-1-phenylpyrazoles. More importantly, the finding that a diverse group of widely used insecticides converge on a common molecular target within mammalian GABR β_3 has important implications for human risk, especially in those individuals with heritable impairments in GABA_AR signaling pathways.

The hypothesis of an imbalance between excitation and inhibition within developing CNS circuits as a common etiological factor contributing to autism susceptibility is supported by several reports of GABR abnormalities in autism. Of particular relevance to the environmental exposures to OC insecticides and 4-alkyl-1-phenylpyrazoles discussed above, is the significantly lower level of GABR β_3 expression measured in postmortem brain samples obtained from children diagnosed with autism, Rett Syndrome and Angelman Syndrome that

may stem from abnormal regulation of DNA methylation by the methyl CpG binding protein MeCP2 (reviewed below by Dr. LaSalle). Autism has been linked to polymorphisms within additional genes that encode GABR, including GABR γ_1 located on 15q11-13 (Vincent *et al.*, 2006; Ashley-Koch *et al.*, 2006). Complex epistatic interactions between genes that encode GABR α_4 and GABR β_1 within 4q12 have also been reported in autistic probands (Ma *et al.*, 2005). Collectively these data implicate GABR dysregulation as a major contributor to imbalances between excitation and inhibition in the autistic brain and may provide the best lead for studying gene \times environment interactions that enhance autism susceptibility.

Roberts and coworkers recently published the results of a study that examined possible association between maternal residence near agricultural pesticide applications during key periods of gestation and development of autism (Roberts *et al.*, 2007). Of nearly 250 hypotheses tested, children of mothers living within 500 meters of field sites with the highest non-zero quartile of organochlorine (OC) (primarily endosulfan and dicofol) poundage had a risk factor for autism that was 6.1 times higher than that of mothers not living near agricultural fields. Autism risk increased with poundage of OC applied and decreased with distance from field sites (Roberts *et al.*, 2007). Although small in sample size, these new findings underscore the critical need to understand how heritable imbalances in GABR signaling and their associated pathways influence susceptibility to chemicals that are known to directly target GABA receptors, especially GABR β_3 , during gestation and postnatal development. However, predicting the functional consequences of exposure to these chemicals is difficult because we do not have sufficient scientific data regarding the specificity and functional outcome of interactions between toxicants that target GABR and the sixteen known GABR subunits that oligomerize as heteropentamers or homotetramers to give rise to the diverse functional roles of these receptors in regulating CNS excitability. Development of relevant models in mice will be essential to understanding the mechanisms by which complex gene \times environment interaction at the level of GABR signaling influence autism risk and to better predict susceptible endophenotypes.

Chemicals that interfere with calcium (Ca²⁺) signaling

Changes in localized and global intracellular Ca²⁺ concentration represent one of the most common ways in which cells regulate cell cycle, terminal differentiation, migration, and death. Moreover intracellular Ca²⁺ orchestrates hundreds, if not thousands, of biochemical processes essential for metabolism, transport, secretion, and regulation of gene transcription and translation in most mammalian cell types. Thus, Ca²⁺ is a fundamental regulator of most biological processes. Although Ca²⁺-regulated processes vary according to cell type and the chemical and physical environment in which the cell finds itself, significant progress has been made toward understanding the mechanisms by which cells generate spatially and temporally segregated Ca²⁺ signals that are context-specific (Berridge 2006). Both the nAChR and GABR that are implicated in autism contribute directly or indirectly to the regulation of Ca²⁺-dependent pathways and are themselves regulated by the Ca²⁺ dependent process. For example, GABR activation in neurons initiates at least two distinct signal transduction pathways, one in which the Cl⁻ current is activated and another which involves the elevation of intracellular Ca²⁺ through functional coupling with voltage activated Ca²⁺ channels (Lyons *et al.*, 2001). Moreover, several of the candidate genes for autism encode proteins whose primary role is to generate intracellular Ca²⁺ signals or are themselves tightly regulated by local fluctuations in Ca²⁺ concentrations (Pessah and Lein, 2008).

A large number of priority chemicals of concern to human environmental health have been shown to affect the integrity of cellular Ca²⁺ signals. Prominent examples are the polyaromatic hydrocarbons that mediate their toxicity through the arylhydrocarbon receptor (AhR), such as dioxin, or through selective interactions with ryanodine receptors (RyR) in the brain (Zimanyi

and Pessah, 1991), such as non-coplanar polychlorinated biphenyls (PCBs). Interactions of non-coplanar PCBs with RyR greatly sensitize the release of Ca^{2+} from the microsomal intracellular stores (Wong *et al.*, 1997; Pessah *et al.*, 2006), and are likely to contribute to imbalances in excitatory and inhibitory neurotransmission and the abnormal development of brain circuitry (Kenet *et al.*, 2007; and review below by Lein). Sensitization of RyRs by PCBs has been shown to be responsible for a host of toxicological outcomes *in vitro* including amplification of NMDA-mediated excitation and Ca^{2+} /caspase mediated apoptosis (Gafni *et al.*, 2004; Wong *et al.*, 2001; Howard *et al.*, 2003). RyRs are physically and functionally linked to voltage gated Ca^{2+} channels at the surface of the neuron where they form Ca^{2+} release units responsible for generating microdomains of signaling (Ca^{2+} microdomains) (Berridge 2006). Of relevance to autism susceptibility, a gain-of-function missense mutation in the L-type Ca^{2+} channel $\text{Ca}_V1.2$ causes Timothy Syndrome which has a 60% autism rate (Splawski *et al.*, 2004). Could inherent abnormalities in Ca^{2+} signaling pathways represent a major point of convergence in the pathobiology of autism? Could environmental factors that dysregulate the spatial and temporal fidelity of Ca^{2+} microdomains amplify inborn weaknesses in generating and decoding Ca^{2+} -dependent processes leading to heightened susceptibilities to autism risk and severity and its associated co-morbidities? Considering that defective neuronal connectivity is now being considered a common etiological feature in most forms of autism, and its phenotypic manifestation is closely linked to imbalances in brain circuitry, then the hypothesis of gene \times environment as it relates to Ca^{2+} signals requires more attention.

II. Developmental Neuroendocrine Effects of Polychlorinated Biphenyls (PCBs): Parallels with Attention Deficit Hyperactivity Disorder (ADHD). R.F. Seegal, Wadsworth Center, Albany, NY, USA

ADHD is the most commonly diagnosed neuropsychiatric/neurological disorder in children (McGough, 2005) and is characterized by behavioral hallmarks including attentional deficits, impulsivity and motor over-activity (Himelstein *et al.*, 2000; Biederman, 2005). The incidence of this disorder ranges from 2 to 16% (Goldman *et al.*, 1998), indicating the difficulties in diagnosis, the potential co-morbidity with other neuropsychiatric/neurological disorders and the likelihood that ADHD is a disorder that includes multiple and perhaps different core deficits (Pennington, 2005). This diversity of diagnoses and symptoms of ADHD complicate studies whose aims are to determine the underlying causes and mechanisms responsible for this common disorder.

We will first briefly describe the neurochemical and neuroendocrine factors shown to be either associated with or acting as etiologic factors in ADHD and then describe the rationale for examining the potential roles that polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) play in this disorder.

The Role of Central Catecholamines in ADHD

There is considerable evidence that dysfunctions in central catecholamines are intimately associated with ADHD with the most direct evidence based on the knowledge that medications most successful in ameliorating ADHD symptoms increase the release of dopamine (DA) and norepinephrine (NE) (Solanto, 2002; Madras *et al.*, 2005). These findings suggests that hypo-functionality of central catecholamines is a major factor contributing to the behavioral phenotype (Russell, 2003). A likely explanation for this catecholamine hypo-functionality is derived from imaging studies conducted in children and adults diagnosed with ADHD. Thus, Cheon *et al.* (2003) report increased dopamine transporter (DAT) densities in basal ganglia. Such an increase in DAT densities, assuming no change in transporter function, would result in a decrease in synaptic concentrations of DA and subsequent alterations in postsynaptic DA receptor regulation in the basal ganglia and prefrontal cortex (PFC). Alternatively, the increases

in DAT densities may represent an up-regulation of DAT induced by reductions in synaptic concentrations of DA and NE due to processes not yet completely understood.

Laboratory studies also support the hypothesis that alterations in central catecholamines are associated with behavioral changes reminiscent of those seen in ADHD. The most commonly studied rodent model of ADHD is the spontaneous hypertensive rat (SHR) which demonstrates many of the behavioral deficits seen in ADHD (Sagvolden *et al.*, 2005). Most importantly, there are also dysfunctions in central catecholamines, including impaired release of catecholamines in PFC and striatum (Davids *et al.*, 2003), increased tissue concentrations of DA receptors, decreased NE function in PFC and striatum (Viggiano *et al.*, 2004) and increased DAT densities (Watanabe *et al.*, 1997).

The DAT knockout mouse has also been proposed as an animal model of ADHD (Gainetdinov and Caron, 2003). This knockout exhibits hyperactivity, the inability to inhibit ongoing behaviors and cognitive and sensorimotor gating deficits (Madras *et al.*, 2005) that mimic some of the deficits seen in ADHD. Because DAT function is virtually absent, it has been suggested that DA **hyper**-functionality, rather than **hypo**-functionality, may contribute to ADHD (Gainetdinov and Caron, 2003). However, these authors, who developed the DAT knockout model, show that these mice adapt to reductions in DAT by reducing tyrosine hydroxylase (the rate-limiting enzyme responsible for the synthesis of DA), the number of vesicular DA storage sites and postsynaptic DA receptor densities. Thus, it is likely that the DAT knockout mouse also demonstrates functional hypo-dopaminergic tone, perhaps simplifying the task of understanding the role that altered central DA and NE function plays in the etiology of ADHD.

The Role of Thyroid Hormone Deficiencies in ADHD

The impetus for study of possible relationships between thyroid hormone dysfunctions and ADHD was provided by Hauser *et al.* (1993) who demonstrated that a majority of children with resistance to thyroid hormone (RTh) exhibited ADHD-like symptoms. Recent studies, however, have shown that children with RTh exhibit more profound deficits, including developmental delays, than seen in children diagnosed with ADHD (Stein *et al.*, 1995). Nevertheless, examination of the relationships between mild hypothyroidism and ADHD has proven fruitful. Stein and Weiss (2003) have shown that thyroid hormone concentrations, in children referred to a clinic specializing in learning and behavioral problems, are associated with attentional deficits and hyperactivity and that the ADHD subtype, primarily inattentive, was associated with reductions in free thyroid hormone concentrations.

The importance of alterations in thyroid hormone function during development in the etiology of ADHD extends beyond evidence of alterations in behavior. Auso *et al.* (2004) and Dulce-Madeira *et al.* (1990) demonstrate that reductions in thyroid hormone function during development, induced respectively by either methimazol (MMI) or propylthiouracil (PTU), reduce cortical neurogenesis and alter its cytoarchitecture, including reductions in PFC volume. These findings are particularly relevant to ADHD since imaging studies carried out in children and adults diagnosed with ADHD demonstrate significant reductions in cortical, basal ganglia and callosal volumes (Seidman *et al.*, 2005). Finally, Evans *et al.* (1999) have shown that experimentally induced hypothyroidism results in reductions in central catecholamines and neurotransmitter metabolic enzymes, raising the important, but as yet, incompletely addressed question of the relationships between developmental exposure to PCBs and PBDEs, fetal and maternal hypothyroidism and reductions in fetal and offspring central catecholamines.

Taken together, the above results suggest a surprisingly consistent image of the neurochemical and endocrine changes associated with ADHD: a positive response to psychostimulants that increase neuronal release of DA and NE; imaging studies that indicate significant increases in basal ganglia DAT densities that are likely to result in a decrease in synaptic concentrations of

DA; genetic studies demonstrating associations between the DAT and postsynaptic DA receptors including the DA D4 receptor, present at high concentrations in the PFC (Heiser *et al.*, 2004); and evidence of associations between thyroid hormone reductions and ADHD.

Why PCBs?

PCBs are widespread persistent environmental contaminants shown to affect cognitive function in infants and children exposed during development (Jacobson and Jacobson, 1996). Most germane to this discussion are recent findings from Jacobson and Jacobson (2003) and Stewart *et al.* (2005) demonstrating that such exposures lead to deficits in attentional domains, including hyperactivity and impulsivity.

The extensive literature demonstrates that PCBs reduce dopamine and norepinephrine concentrations in a variety of experimental paradigms, including cells in culture (Shain *et al.*, 1991), the brains of adult rodents and non-human primates (Seegal *et al.*, 1991), and the brains of mice and rats exposed during development, including the PFC and basal ganglia (Seegal *et al.*, 1997). In addition to reductions in concentrations of central catecholamines there is also convincing evidence that PCBs inhibit both the dopamine transporter and the vesicular monoamine transporter (Mariussen and Fonnum, 2001; Bemis and Seegal, 2004)—transporters also shown to be altered in ADHD and experimental models of ADHD (Gainetdinov and Caron, 2003; Madras *et al.*, 2005). Although it may seem counterintuitive that DAT inhibition could result in catecholaminergic hypofunction, there is evidence that such inhibition results in a reduction in synaptic catecholamine levels. Thus, Seegal *et al.* (2002) have shown, using *in vivo* microdialysis, that exposure of the adult rat to PCBs for periods longer than three days results in significant reductions in extracellular concentrations of striatal DA.

In summary, there appears to be convincing evidence that PCBs and PBDEs induce changes in neuroendocrine function in laboratory animals reminiscent of those seen in humans diagnosed with ADHD. Understanding the mechanisms by which PCBs and PBDEs induce these changes following developmental exposure will aid in understanding the etiology of this widely diagnosed disorder.

III. Polychlorinated Biphenyls (PCBs) Modulate the Development of Neuronal Connectivity Pamela J. Lein, CROET, Oregon Health & Science University; Portland, OR 97239

PCBs are persistent widespread environmental contaminants, and high residue levels are still detected in human tissues (Humphrey *et al.*, 2000; DeCaprio *et al.*, 2005; Park *et al.*, 2007). Epidemiological data indicate that PCBs negatively impact neuropsychological function in exposed children (Schantz *et al.*, 2003; Carpenter, 2006), and experimental animal studies confirm that developmental PCB exposure causes cognitive and psychomotor deficits (Mariussen and Fonnum, 2006). PCBs disrupt thyroid hormone function (Hagmar, 2003; Zoeller, 2007), perturb dopaminergic neurotransmission (Seegal, 1996; Mariussen and Fonnum, 2006) and increase intracellular Ca²⁺ levels in neurons via several mechanisms (Kodavanti, 2005; Mariussen and Fonnum, 2006), including ryanodine receptor (RyR) activation (Pessah and Wong, 2001). The relevance of these molecular effects to PCB-induced neurobehavioral deficits has been difficult to establish, in part because neurodevelopmental events targeted by PCBs have yet to be identified. We are investigating the hypothesis that PCBs disrupt normal patterns of neuronal connectivity via effects on dendritogenesis. This hypothesis is derived from observations that: 1) subtle perturbations of temporal or spatial aspects of dendritic growth are associated with altered behavior in experimental models (Berger-Sweeney and Hohmann, 1997; Barone *et al.*, 2000); 2) in humans, aberrations in dendritic structure are thought to contribute to functional deficits observed in a variety of

neurodevelopmental disorders (Jagadha and Becker, 1989; Huttenlocher, 1991; Rubenstein and Merzenich, 2003; Zoghbi, 2003); and 3) both thyroid hormone (Kapfhammer, 2004) and intracellular Ca^{2+} (Lohmann *et al.*, 2005; Redmond and Ghosh, 2005) influence neuronal connectivity via dynamic control of dendritic structure.

To test this hypothesis, we examined spatial learning and memory in parallel with dendritic growth and plasticity of cerebellar Purkinje cells and neocortical pyramidal neurons in weanling rats exposed to the PCB mixture Aroclor 1254 (A1254) in the maternal diet at 1- or 6-mg/kg/d throughout gestation and lactation. These doses and routes of exposure are similar to those reported to generate PCB levels in rat adipose tissue and brains within the range of levels observed in humans (Bruun *et al.*, 1990; Stellman *et al.*, 1998; Hany *et al.*, 1999; DeCaprio *et al.*, 2005). These doses did not cause maternal or fetal toxicity as assessed by body weight of pregnant and lactating dams, litter size, and weight gain in pups. However, developmental A1254 exposure impaired performance in the Morris water maze and altered dendritic morphology of both cerebellar Purkinje cells and neocortical pyramidal neurons, enhancing dendritic growth in untrained animals but attenuating or reversing experience-dependent dendritic growth in maze-trained littermates. Both behavioral and structural deficits were more pronounced in the 1 versus 6-mg/kg/d A1254 treatment group. Consistent with previous reports (Crofton *et al.*, 2000; Zoeller *et al.*, 2000; Roegge *et al.*, 2004), dose-dependent decreases in serum thyroid hormone were observed in weanlings exposed developmentally to A1254. However, it seems unlikely that the PCB effects on dendritic growth were due to hypothyroxinemia because neonatal hypothyroidism would be expected to decrease basal dendritic growth in cerebellar and cortical neurons (Nicholson and Altman, 1972; Rami *et al.*, 1986; Ruiz-Marcos *et al.*, 1994; Uylings *et al.*, 1994). In contrast, radioligand binding and western blot analyses indicated that developmental A1254 exposure increased basal cerebellar [^3H]ryanodine binding sites and RyR expression, but inhibited training-induced RyR upregulation. Interestingly, the relationship of A1254 dose to A1254 effects on basal and training-induced RyR expression closely paralleled the inverted-dose related effects of developmental A1254 exposure on dendritic morphology. Given the fundamental role of RyR in Ca^{2+} signaling and the critical influence of Ca^{2+} signaling on basal and activity-dependent dendritic growth, these data suggest that PCB effects on RyR could be largely responsible for the effects of developmental PCB exposure on behavior and dendritic morphology that we observed in weanling rats. In support of that conclusion, *in vitro* studies demonstrated that dendritic growth of neocortical pyramidal neurons was influenced by PCB95, a non-coplanar congener that sensitizes ryanodine receptors (RyR), but not by PCB66, a coplanar congener with negligible RyR activity (Pessah *et al.*, 2006).

These data demonstrate that developmental exposure to environmentally relevant levels of PCBs interferes with normal patterns of dendritic growth and plasticity, and suggest that PCB effects on RyR expression and function may mediate this response. The relevance of these findings to autism spectrum disorders is suggested by recent genetic studies implicating genes that encode Ca^{2+} -regulated signaling proteins involved in synapse formation and dendritic growth in autism (Krey and Dolmetsch, 2007) and emerging histological, electrophysiological and functional imaging studies suggesting that autism results from alterations in neuronal connectivity (Zoghbi, 2003; Belmonte and Bourgeron, 2006; Geschwind and Levitt, 2007). Collectively, these observations suggest that PCBs, and in particular non-coplanar PCBs, may be environmental risk factors in autism spectrum disorders and provide important new clues about the possible role of RyR in contributing to environmentally triggered neurodevelopmental deficits. Moreover, they suggest the possibility that exposure to even very low PCB levels could amplify adverse effects in genetically susceptible individuals (Campbell *et al.*, 2006) such as those with heritable deficits in Ca^{2+} signaling.

IV. Epigenetic Influences on Autism Risk: A Role for GABA_A Receptor Dysregulation Janine M. LaSalle, University of California, Davis, CA, 95616, USA

Epigenetics is the study of inheritable and reversible modifications to nucleotides or chromosomes that do not change the genetic sequence but can modify gene expression and phenotype. The importance of epigenetic mechanisms in regulating human brain development has been recently revealed by the discovery of the genetic bases of several human neurodevelopmental disorders (Egger *et al.*, 2000; Hendrich *et al.*, 2001; Zoghbi *et al.*, 2003). Genetic syndromic causes of autism that involve epigenetic mechanisms include Rett syndrome, an X-linked disorder caused by mutations in *MECP2* and encoding methyl CpG binding protein 2 (Amir *et al.*, 1999). Although originally described as a transcriptional repressor of methylated genes, new roles for MeCP2 in chromatin organization and alternative splicing have recently emerged (Horike *et al.*, 2005; Yau *et al.*, 2007; Young *et al.*, 2005). Angelman syndrome is an imprinted disorder caused by maternal 15q11-13 deficiency or mutation of the ubiquitin protein ligase E3 gene, *UBE3A*, that also shares features with both Rett syndrome (RTT) and autism (Hitchins *et al.*, 2004; Lalande and Calciano, 2007; Philippart, 2001; Watson *et al.*, 2001). The demonstration of MeCP2 as a regulator of both *UBE3A* (the Angelman gene) and *GABRB3* (encoding a GABA_A receptor subunit) expression within 15q11-13 has revealed some interesting insights into the genetic and epigenetic pathways common to all three disorders (Hogart *et al.*, 2007; Samaco *et al.*, 2005). More common neurodevelopmental disorders of unknown genetic etiologies such as autism and developmental delays are likely to be caused by a combination of genetic and environmental factors (Lamb *et al.*, 2000; Muhle *et al.*, 2004; Volkmar and Pauls, 2003). Since epigenetic pathways act at the interface between genes and the environment, environmental factors can alter epigenetic patterns and downstream gene expression pathways.

Pervasive developmental disorders classified under the DSM IV criteria include autistic disorder, Asperger's syndrome, RTT, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified (PDD-NOS) (Volkmar and Pauls, 2003). The onset of autistic symptoms in these disorders generally occurs around 1 to 3 years of age and is characterized by stereotyped mannerisms, abnormal preoccupations, lack of pragmatic language and imaginative play, impaired eye gaze, and impaired joint attention (16). Males with autistic disorder outnumber females by around 4:1, while a 12:1 sex bias is observed for Asperger's syndrome (AS) (Muhle *et al.*, 2004). In contrast, RTT is almost exclusively in females because of the X-linked dominant inheritance pattern (Zoghbi, 2003). While twin studies have demonstrated high heritability of autism-spectrum disorders, monozygotic twins are not always concordant for autistic disorder (Bailey *et al.*, 1995). Epigenetic changes are predicted to be a major determinant in differences between monozygotic twins (Fraga *et al.*, 2005).

Maternal duplications of 15q11-13 by interstitial duplications or marker chromosomes are the most common cytogenetic cause of autism, occurring in 1–2% of patients (Schroer *et al.*, 1998). Multiple studies have shown an association of 15q11-13 markers to autism, and GABA_A receptor genes have been identified as strong candidates to explain the occurrence of seizures in 30–35% of autistic patients (Buxbaum *et al.*, 2002; Martin *et al.*, 2000; Menold *et al.*, 2001; Ma *et al.*, 2005). The 15q11-13 locus contains a cluster of imprinted genes, expressed exclusively from one parental chromosome, as well as a cluster of three GABA_A receptor subunit genes that are biallelically expressed (Nicholls *et al.*, 1993). The 15q11-13 GABA_A receptor genes are implicated in AS, as patients with a 15q11-13 deletion have a more severe phenotype, particularly in occurrence of seizures, than do individuals with *UBE3A* point mutations (Minassian *et al.*, 1998). Additionally, the *Gabrb3* knock-out mouse demonstrates

the significance of the 15q11-13 gene as 90% of pups die as neonates, and surviving mice exhibit severe neurological manifestations such as seizures, learning deficits, hyperactivity, and disturbed rest-activity cycles (Homanics *et al.*, 1997; DeLorey *et al.*, 1998). Mouse deletion strains of the syntenic 15q11-13 GABA_A receptor cluster have demonstrated that *Gabrb3* deficiency causes the most severe phenotype, as mice with a deletion encompassing *Gabra5* and *Gabrg3* are phenotypically normal (Culiat *et al.*, 1994).

GABRB3 protein expression is reduced in RTT, caused by mutations in *MECP2* on Xq28 (Samaco *et al.*, 2005). Although *Gabrb3* is biallelically expressed in mouse brain, conflicting data exist regarding the imprinting status of the 15q11-13 GABR genes in humans (Meguro *et al.*, 1997; Bittel *et al.*, 2003; Bittel *et al.*, 2005; Gabriel *et al.*, 1998). A recent study showed that all three GABR genes were biallelically expressed in 21 control brain samples, demonstrating the lack of imprinting in normal human cortex (Hogart *et al.*, 2007). Interestingly, four of eight autism and one of five RTT brain samples showed monoallelic or highly skewed allelic expression of one or more GABR genes, suggesting that epigenetic dysregulation of these genes is common to both disorders. Prader-Willi Syndrome (PWS) and Autism Spectrum (AS) samples with paternal and maternal 15q11-13 deletions revealed a paternal expression bias of *GABRB3*, while RTT brain samples showed a significant reduction in *GABRB3* and *UBE3A*. MeCP2 binds to methylated CpG sites within an intronic sequence of *GABRB3*. Previous studies demonstrated that homologous 15q11-13 pairing in neurons was dependent on MeCP2 and was disrupted in RTT and autism cortexes (Thatcher *et al.*, 2005). Combining these results suggest that MeCP2 acts as a chromatin organizer for optimal expression of both alleles of *GABRB3* in neurons.

Since epigenetic mechanisms act at the interface between genetic and environmental factors that determine phenotype, investigation of epigenetic mechanisms are essential for understanding the multiple interacting etiologies of complex diseases such as autism. Since human populations have a diverse array of genetic and environmental differences, the major challenge is how to define precise epigenetic changes that occur as a result of a single environmental or genetic change and to understand how such changes affect disease risk. Future studies will investigate potential epigenetic changes to *MECP2* and 15q11-13 genes following perinatal exposure to organic pollutants, specifically polybrominated diphenyl ethers used in flame retardants.

V. The Impacts of Maternal Immune Challenge on the Fetal Brain and the Pathological Consequences on Behavior. Benjamin K. Yee, Urs Meyer, and Joram Feldon, Laboratory of Behavioural Neurobiology, ETH Zurich, Switzerland

Maternal infection in prenatal life is a notable risk factor in the development of severe neuropsychiatric disorders in later life, including schizophrenia and autism (reviewed in Brown and Susser, 2002; Arndt *et al.*, 2005; Fatemi, 2005; Brown, 2006; Patterson, 2007). One prevalent hypothesis suggests that infection-induced disruption of early prenatal brain development may predispose the organism for long-lasting structural and functional brain abnormalities, leading to the emergence of psychopathological behavior in adulthood (Gilmore and Jarskog, 1997; Patterson, 2002; Meyer *et al.*, 2007). The feasibility of this causal link (between maternal infection during pregnancy and higher risk of brain and behavioral pathology in the offspring) has received considerable support from several experimental models established in both rats and mice (for a review, see Meyer *et al.*, 2007).

One class of experimental models favors the use of agents that stimulate infection-like immune response in the maternal host, such as polyriboinosinic-polyribocytidilic acid (Poly-I:C, a

synthetic analog of double-stranded RNA) or lipopolysaccharide (LPS, a gram-negative bacteria cell wall product), rather than live pathogens. Administration of Poly-I:C mimics the acute phase response to viral infection, and is highly effective in elevating the levels of pro-inflammatory cytokines and other mediators of inflammation (e.g., Fortier *et al.*, 2004; Meyer *et al.*, 2006b; Cunningham *et al.*, 2007). Systemic Poly-I:C treatment to pregnant mice and rats results in significant and time-limited alterations of pro- and anti-inflammatory cytokine levels in the three relevant compartments of the maternal-fetal interface: the placenta, the amniotic fluid, and the fetus, including the fetal brain (Gilmore *et al.*, 2005; Meyer *et al.*, 2006b, 2008a). The use of prenatal immune challenge by Poly-I:C is thus a valuable experimental approach in studying the long-term consequences of fetal brain inflammation in specific gestation time on subsequent brain and behavioral development.

This approach has been successfully applied by Benjamin Yee, Urs Meyer and Joram Feldon from the Swiss Federal Institute of Technology (ETH) Zurich in mice, providing a comprehensive characterization of the neuropathological and psychopathological consequences of prenatal immune activation. Their efforts also included experiments designed to identify distinct neuroimmunological factors that may be critical in shaping the risk for brain and behavioral dysfunctions emerging after *in utero* exposure to infection. Their results highlighted the importance of 1) the relative prenatal and postnatal maternal contributions to the behavioral and neurochemical dysfunctions after prenatal immune activation, 2) the precise timing of prenatal immune challenge on the specificity of postnatal neuropathology and psychopathology, and 3) the influence of the anti-inflammatory genetic background in the association between prenatal immune activation and adult behavioral pathology.

In order to dissect the relative prenatal and postnatal contributions in the infection model of schizophrenia and related disorders, pregnant mice were exposed to PolyI:C or vehicle treatment in early/middle pregnancy (gestation day 9), and offspring born to Poly-I:C- and vehicle-treated dams were then systematically cross-fostered to surrogate rearing mothers, which had experienced either inflammatory or vehicle treatment during pregnancy. The findings from these experiments demonstrate that neuropathological and psychopathological abnormalities emerging after prenatal PolyI:C-induced immune challenge are largely attributable to prenatal rather than postnatal maternal effects on the offspring (Meyer *et al.*, 2006c, 2008b). Regardless of whether the neonates were reared by immune-challenged or control surrogate mothers, multiple brain and behavioral dysfunctions emerged following prenatal Poly-I:C exposure. This strongly indicates that infection-induced disruption of fetal brain development predisposed the offspring to the emergence of psychopathology in later life, and the normalization of neonatal rearing condition provided no protection. Notwithstanding, these cross-fostering experiments further showed that being reared by an immune-challenged surrogate mother was sufficient to induce distinct forms of adult psychopathology and neuropathology (Meyer *et al.*, 2006c, 2008b). The responsible postnatal factors, including post-partum maternal behavior, critical for the latter effects clearly warrant further specification. This would reveal additional environmental factors in early life contributing to later life's vulnerability to psychiatric disorders.

Another series of experiments was designed to evaluate the influence of the timing of maternal immune challenge on the emergence of brain and behavioral dysfunctions in the resulting offspring (Meyer *et al.*, 2006a,b, 2008c). These experiments demonstrated that the precise timing of prenatal immune activation is a crucial determinant of the specificity of both the structural and functional brain abnormalities in later life (see Meyer *et al.*, 2007). Specifically, prenatal PolyI:C-induced immune challenge in early/middle gestation (gestation day 9 in mice) led to a pathological profile characterized by suppressed exploratory behavior, abnormalities in selective associative learning in the form of latent inhibition disruption and loss of the US-pre-exposure effect, deficiency in sensorimotor gating in the form of impaired prepulse

inhibition, enhanced sensitivity to the indirect dopamine receptor agonist amphetamine and (to a lesser extent) the non-competitive NMDA-receptor blocker, dizocilpine (MK-801), as well as deficient spatial working memory function when the temporal demand on memory retention was high (Meyer *et al.*, 2005, 2006a, Meyer *et al.*, b, 2008c). On the other hand, prenatal immune challenge in late gestation (gestation day 17) led to a partially overlapping symptom profile, which comprised perseverative behavior retarding reversal learning, a severe working memory deficit, potentiated response to acute systemic amphetamine and dizocilpine treatment, and the loss of the US-pre-exposure effect (Meyer *et al.*, 2006a,b; 2008c). Hence, some of the identified pathological traits are clearly restricted to the symptom cluster associated with prenatal immune activation in early/middle or late gestation (e.g. latent inhibition deficiency and retardation in reversal learning), whereas others are common to both symptom clusters (e.g., potentiation of amphetamine sensitivity). Existing data further indicate that prenatal immune challenge in early/middle gestation is likely to exert a more severe impact on neurodevelopment at least with respect to the resulting behavioral abnormalities emerging in adulthood, in comparison to identical immune challenge conducted in late gestation (Meyer *et al.*, 2007).

The link between prenatal immune challenge and postnatal brain dysfunctions may appear to be critically modulated by the precise pattern of cytokine reactions taking place at the maternal-fetal interface. To test this hypothesis, Meyer *et al.* (2008a) compared the psychopathological consequences of prenatal immune activation by Poly-I:C in wild-type mice and transgenic mice constitutively over-expressing interleukin (IL)-10 in macrophages. IL-10 is a cytokine with strong anti-inflammatory and immuno-suppressive functions; it limits production and/or secretion and biological activities of many inflammatory molecules in both acute and chronic conditions (Moore *et al.*, 2001; Murray, 2006). If the emergence of postnatal brain abnormalities after prenatal infection and/or inflammation depends primarily on a shift of increased pro-inflammatory cytokines levels in the fetal brain (Gilmore and Jarskog, 1997), then the impact of prenatal infection ought to be minimal if this is expressed against a background of enhanced IL-10 production.

This prediction has been confirmed by Meyer *et al.* (2008a) who showed that genetically engineered peripheral over-expression of the anti-inflammatory cytokine IL-10 was sufficient to prevent the emergence of multiple behavioral and psychopharmacological abnormalities in the adult offspring associated with prenatal immune challenge by Poly-I:C (Meyer *et al.*, 2008a). To their surprise, however, in the absence of a discrete prenatal inflammatory stimulus, IL-10 over-expression during prenatal development was in itself sufficient to induce specific behavioral abnormalities in the adult offspring (Meyer *et al.*, 2008a). One startling implication is that the development of normal adult brain functions may be critically influenced by the precise balance between pro- and anti-inflammatory cytokines in prenatal life. Accordingly, shifts of the balance towards either pro-inflammatory or anti-inflammatory cytokine species would precipitate adult behavioral pathology, whereas the concomitant induction of both cytokine classes in the fetal brain may nullify each other's long-term negative influences on brain and behavioral development. This account is consistent with the antagonistic properties of distinct cytokine classes in the peripheral immune system (Moore *et al.*, 2001; Murray, 2006) and suggests that the precise effects of cytokines on early neurodevelopmental processes crucially depend on their identity.

In summary, the findings from experimental models of prenatal immune activation in mice show that early life experience in the form of a discrete immunological manipulation can cumulate into specific brain and behavioral pathology in early adolescence and/or adulthood. The data thus support the biological and neuroimmunological plausibility for a causal link from maternal infection and/or inflammation during pregnancy to higher risks for brain pathology in the offspring. Given that Poly-I:C readily mimics the acute immune response to viral

infection, the identified long-term effects of prenatal Poly-I:C-induced immune activation may be of particular relevance for validating the plausibility of the epidemiological association between prenatal infection with viral pathogens and enhanced risk for postnatal brain disorders, including schizophrenia and autism (Brown and Susser, 2002; Arndt *et al.*, 2005; Fatemi, 2005; Brown, 2006; Patterson, 2007). The data from the laboratory of Benjamin Yee, Urs Meyer and Joram Feldon have further identified that this relationship is 1) attributable to prenatal rather than postnatal maternal effects on the offspring, 2) critically influenced by the precise timing of maternal immune response, and 3) dependent on the immunological background (genetically determined or otherwise) of the infected maternal host.

VI. Maternal autoantibodies: developmental neurotoxicants of autism risk?

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Autism Spectrum Disorders (ASD) are neurodevelopmental disorders characterized by impairments in social interaction, verbal and nonverbal communication, and stereotyped behaviors and interests (Lord *et al.*, 2000a). ASD encompasses a broad range of phenotypes and diagnosis is currently based solely on behavioral criteria (Lord *et al.*, 2000b). The etiology of ASD is not well understood, though it likely involves both genetic and environmental factors (Volkmar and Pauls, 2003). Although the age of symptom onset would indicate a pre-natal or early post-natal etiology, to date, no early biological markers have been identified for this spectrum of disorders. Such markers would allow for earlier identification and therapeutic intervention, which could significantly improve the prognosis for ASD patients (Aman and Langworthy, 2000) and contribute to the development of prevention strategies.

Immune system dysregulation has been reported in ASD in several studies (Warren *et al.*, 1987, Warren *et al.*, 1990, van Gent *et al.*, 1997, Fiumara *et al.*, 1999, Jyonouchi *et al.*, 2005, Ashwood *et al.*, 2006, Molloy *et al.*, 2006). Systemic immunologic aberrations in individuals with autism have often been associated with autoimmunity; in particular, the generation of antibodies reactive against brain and CNS proteins (Todd *et al.*, 1988, Singh *et al.*, 1993, Singh *et al.*, 1997a, Singh *et al.*, 1997b, Connolly *et al.*, 1999, Singh *et al.*, 2002, Ashwood and Van de Water, 2004, Singh and Rivas, 2004, Cohly and Panja, 2005, Connolly *et al.*, 2006, Cabanlit *et al.*, 2007, Wills *et al.*, 2007).

In addition to the presence of autoantibodies in children with autism, a few studies suggest the presence of autoantibodies to brain proteins in the blood of mothers of children with autism. The transplacental passage of maternal IgG isotype antibodies has long been known as a mechanism for fetal immune instruction (Garty *et al.*, 1994) and protection (Simister, 2003, Harris *et al.*, 2006). However, autoantibodies that react to fetal 'self'-proteins can also cross the placenta, and potentially impact fetal development.

For example, Dalton *et al.* (Dalton *et al.*, 2003) demonstrated maternal IgG antibody reactivity to adult rat cerebellar Purkinje cells in a mother of multiple children with ASD. These authors also reported the presence of behavioral deficits in the pups of a mouse injected during gestation with her serum (Dalton *et al.*, 2003). Similarly, Zimmerman *et al.* (Zimmerman *et al.*, 2007) recently reported differing patterns of serum immunoreactivity to pre-natal rat brain between mothers of children with autism and mothers of control children. This study also demonstrated that immunoreactivity persisted in maternal circulation for up to 18 years post-delivery.

Our laboratory has recently noted the presence of autoantibodies to human fetal brain proteins at 37kDa and 73kDa exclusively in the plasma of mothers of children with autism compared with control mothers (Braunschweig *et al.*, 2007). In this study, maternal plasma was collected on average 3.5 years after the birth of the study child. Figure 1 illustrates the various band patterns noted in the plasma from mothers of children with autism. Maternal reactivity to a

protein at approximately 37kDa was observed in the plasma of 16/61 mothers of AU children (26.2%) (Figure 1) compared with 1/40 mothers of developmentally delayed (DD) children (2.5%; $p=0.0023$), and 5/62 mothers of typically developing (TD) children (8.1%; $p=0.0086$). Furthermore, the presence of the 37kDa band yielded a significantly elevated odds ratio of 5.69 (95% confidence interval: 2.09 – 15.51) when compared with the TD group. Of particular note, reactivity against proteins at both 37kDa and 73kDa was observed only in mothers of AU children, yielding highly significant statistical differences between mothers of AU children and mothers of TD children (7/61 vs. 0/62; $p=0.0061$) and mothers of DD children (0/40; $p=0.0401$). This pattern was not observed when only the 73kDa band was present. The presence of these bands did not correlate with maternal age or history of autoimmune disease, nor with birth order or IQ of the index child.

When band prevalence was analyzed based on the pattern of clinical onset of autistic behaviors in children, 6/7 (86%) of the AU mothers that exhibited reactivity to the pair of bands at 37kDa and 73kDa had children with the regressive phenotype. Moreover, this association was also evident regarding reactivity to the 37kDa and 73kDa bands separately. In contrast, the antibody response of the mothers of early onset children was only significantly different from the TD and DD groups for the 37kDa band alone. Finally, no association was observed between the presence of autoreactivity to fetal brain antigens and history of autoimmune disease in the maternal populations at the time of blood draw.

Interestingly, no case-control differences were observed in reactivity to kidney, duodenum or adult brain proteins. This finding suggests the possibility that the autoreactivity described herein is targeted towards proteins expressed exclusively, or at substantially higher levels, during fetal brain development. Given the rather small number of comparison children with non-ASD developmental delays included in the present investigation, further study is necessary to determine the specificity of our findings to autism.

These data provide evidence for an association between the presence of maternal immune system biomarkers and a diagnosis of autism in a subset of children. The presence of specific anti-fetal brain antibodies in the circulation of mothers during pregnancy may be a potential trigger that, when paired with genetic susceptibility, is sufficient to induce a downstream effect on neurodevelopment leading to autism. At present, we are investigating maternal plasma reactivity against fetal brain in a prospective cohort to determine the effect of the gestational autoantibody profile as it relates to an outcome of autism. Furthermore, work is currently underway to both replicate these findings in a larger cohort, and to determine the protein targets of these antibodies, the identification of which will allow us to better understand potential pathogenic mechanisms as well as create specific screening assays.

VII. Examination of Thimerosal Effects in Neonatal SJL/J Mice at Vaccination-Associated Exposure Levels. Robert F. Berman, University of California, Davis, CA, 95616, USA

Thimerosal (sodium ethyl mercury thiosalicylate) is an antimicrobial preservative used since the 1930's in numerous vaccines and medicinal preparations. Ethylmercury poisoning has occurred in humans, resulting in renal and neurotoxicity in the affected populations (Cinca *et al.*, 1980; Damluji 1962; Hilmy *et al.*, 1976; Zhang 1984). However, the possible effects of exposure to lower levels of ethyl mercury, such as those levels associated with immunization with thimerosal-preserved vaccines, are poorly understood. Initial risk assessments for ethylmercury were based on studies of oral methylmercury toxicity (Clarkson *et al.*, 2003; Myers and Davidson 2000); however, recent data indicate that the kinetics of tissue disposition and metabolism differ substantially for these two forms of organic mercury (Burbacher *et al.*,

2005; Clarkson and Magos 2006; Harry *et al.*, 2004; Magos 2003; Magos *et al.*, 1985; Pichichero *et al.*, 2002; Stajich *et al.*, 2000; Suzuki 1973), making the assumptions underlying a risk assessment different for each form of mercury. Concern remains about the possible contribution of exposure to low levels of ethylmercury via childhood vaccinations with thimerosal-preserved vaccines to the etiology of neurodevelopmental disorders, including autism (Bernard *et al.*, 2002).

There is no direct evidence at the present time supporting an association between thimerosal exposure and autism (Davidson *et al.*, 2004; Stratton 2001; Thompson *et al.*, 2007), and the majority of epidemiological studies published to date have not supported such a relationship. However, a study by Hornig *et al.* (Hornig *et al.*, 2004) examined the neurotoxicity of thimerosal in SJL/J mice and suggested that toxicity may be dependent on an organism's underlying immune status. Neonatal injections of thimerosal in SJL/J mice produced abnormal brain development, delayed growth, and altered locomotor behavior. SJL/J mice have the mouse major histocompatibility complex H-2^s associated with increased susceptibility to autoimmunity, and develop autoantibodies, including antinucleolar antibodies, to fibrillar protein (Havarinasab *et al.*, 2005) when exposed to high levels of methyl and ethylmercury (Havarinasab *et al.*, 2005; Havarinasab and Hultman 2005; Hultman and Hansson-Georgiadis 1999). Thimerosal effects were not found in C57BL/6J (H-2^b) or Balb/c (H-2^d) mice that are less susceptible to autoimmunity. Hornig *et al.* (Hornig *et al.*, 2004) suggested that the H-2^s haplotype, and altered immune system function in general, might confer heightened susceptibility to neonatal thimerosal exposures in mice. Although antinucleolar antibodies have not been found in autistic children (Singh and Rivas 2004), the findings in SJL/J mice raised the possibility that children with altered immune system function could be particularly vulnerable to the neurotoxicity of thimerosal, and that the SJL/J mouse strain may provide a sensitive model for thimerosal developmental neurotoxicity studies. These findings are also intriguing because, if confirmed, they would mean that the neurotoxicology of thimerosal, and possibly other forms of mercury, would need to be recast within the framework of immune system status and function. Thus, we reexamined thimerosal neurotoxicity in SJL/J mice. Specifically, we examined the somatic growth, locomotor behavior, and the structure of the hippocampus of SJL/J mice following neonatal thimerosal injections, using experimental procedures that closely followed the original experimental procedures in the Hornig *et al.* (Hornig *et al.*, 2004) study. As an extension of this previous study we also included a higher mercury dose (i.e., 10 times higher), measured tissue mercury levels (blood, brain and kidney), used unbiased stereological techniques for assessing the numbers of hippocampal CA1 pyramidal and dentate granule cell numbers, and included tests of social interaction, sensory gating and anxiety. These behaviors were selected because they assess behavioral domains that are considered relevant to core deficits in neurodevelopmental disorders such as autism (Crawley 2004; Ricceri *et al.*, 2007).

Neonatal SJL/J mice were injected with thimerosal, with and without combined HiB & DTaP vaccines. Injections modeled childhood vaccination schedules, with mice injected on postnatal days 7, 9, 11 and 15 with 14.2, 10.8, 9.2 and 5.6 µg/kg mercury from thimerosal, respectively, or vehicle. Additional groups received vaccine only or a 10-times higher thimerosal+vaccine dose (Berman *et al.*, 2008). Although low levels of mercury were detected in blood, brain and kidneys 24 hr following the last thimerosal injection, survival, body weight, indices of early development (negative geotaxis, righting) and hippocampal morphology were not affected.

Performance was unaffected in behavioral tests selected to assess behavioral domains relevant to core deficits in neurodevelopmental disorders such as autism (i.e., social interaction, sensory gating, anxiety). In an open-field test the majority of behaviors were unaffected by thimerosal injection, although thimerosal-injected female mice showed increased time in the margin of an open field at 4 weeks of age. Considered together our recent results did not indicate pervasive

developmental neurotoxicity following vaccine-level thimerosal injections in SJL mice (Berman *et al.*, 2008).

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References

- Aman MG, Langworthy KS. Pharmacotherapy for Hyperactivity in Children with Autism and Other Pervasive Developmental Disorders. *J Autism Dev Dis* 2000;30:451–519.
- American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders DMS-IV-TR (Text Revision). Washington D.C: American Psychiatric Association; 2000.
- Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked *MECP2*, encoding methyl- CpG-binding protein 2. *Nat Genet* 1999;23:185–8. [PubMed: 10508514]
- Arndt TL, Stodgell CJ, Rodier PM. The teratology of autism. *Int J Dev Neurosci* 2005;23:189–199. [PubMed: 15749245]
- Ashley-Koch AE, Mei H, Jaworski J, Ma DQ, Ritchie MD, Menold MM, DeLong GR, Abramson RK, Wright HH, Hussman JP, Cuccaro ML, Gilbert JR, Martin ER, Pericak-Vance MA. An analysis paradigm for investigating multi-locus effects in complex disease: examination of three GABA receptor subunit genes on 15q11–q13 as risk factors for autistic disorder. *Ann Hum Genet* 2006;70:281–292. [PubMed: 16674551]
- Ashwood P, Van de Water J. Is autism an autoimmune disease? *Autoimmun Rev* 2004;3:557–562. [PubMed: 15546805]
- Ashwood P, Wills S, Van de Water J. The immune response in autism: a new frontier for autism research. *J Leukoc Biol* 2006;80:1–15. [PubMed: 16698940]
- Ausó E, Lavado-Autric R, Cuevas E, Escobar del Rey F, Morreale de Escobar G, Berbel P. A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocortigenesis alters neuronal migration. *Endocrinology* 2004;145:4037–4047. [PubMed: 15087434]
- Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M. Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol Med* 1995;25:63–77. [PubMed: 7792363]
- Barone S Jr, Das KP, Lassiter TL, White LD. Vulnerable processes of nervous system development: a review of markers and methods. *Neurotoxicology* 2000;21:15–36. [PubMed: 10794382]
- Belmonte MK, Bourgeron T. Fragile X syndrome and autism at the intersection of genetic and neural networks. *Nat Neurosci* 2006;9:1221–1225. [PubMed: 17001341]
- Berger-Sweeney J, Hohmann CF. Behavioral consequences of abnormal cortical development: insights into developmental disabilities. *Behav Brain Res* 1997;86:121–142. [PubMed: 9134147]
- Bemis JC, Seegal RF. PCB-induced inhibition of the vesicular monoamine transporter predicts reductions in synaptosomal dopamine content. *Toxicol Sci* 2004;80:288–95. [PubMed: 15115888]
- Biederman J. Attention-deficit/hyperactivity disorder: a selective overview. *Biol Psychiatry* 2005;57:1215–20. [PubMed: 15949990]
- Bittel DC, Kibiryeva N, Talebizadeh Z, Butler MG. Microarray analysis of gene/transcript expression in Prader-Willi syndrome: deletion versus UPD. *J Med Genet* 2003;40:568–74. [PubMed: 12920063]
- Bittel DC, Kibiryeva N, Talebizadeh Z, Driscoll DJ, Butler MG. Microarray analysis of gene/transcript expression in Angelman syndrome: deletion versus UPD. *Genomics* 2005;85:85–91. [PubMed: 15607424]
- Bloomquist JR. Chloride channels as tools for developing selective insecticides. *Arch Insect Biochem Physiol* 2003;54:145–156. [PubMed: 14635176]
- Braunschweig D, Ashwood P, Hertz-Picciotto I, Croen L, Hansen R, Pessah IN, Van de Water J. Maternal serum antibodies to fetal brain in autism. *Neurotoxicology*. 2007In press

- Brown AS. Prenatal infection as a risk factor for schizophrenia. *Schizophr Bull* 2006;32:200–202. [PubMed: 16469941]
- Brown AS, Susser ES. In utero infection and adult schizophrenia. *Ment Retard Dev Disabil Res Rev* 2002;8:51–57. [PubMed: 11921387]
- Brunn H, Georgii S, Prucha J. Polychlorinated biphenyls (PCB) in human adipose tissue. *Z Lebensm Unters Forsch* 1990;190:108–111. [PubMed: 2106759]
- Buxbaum JD, Silverman JM, Smith CJ, Greenberg DA, Kilifarski M, Reichert J, Cook EH, Fang Y, Song CY, Vitale R. Association between a GABRB3 polymorphism and autism. *Mol Psychiatry* 2002;7:311–316. [PubMed: 11920158]
- Cabanlit M, Wills S, Goines P, Ashwood P, Van de Water J. Brain-specific autoantibodies in the plasma of subjects with autistic spectrum disorder. *Ann N Y Acad Sci* 2007;1107:92–103. [PubMed: 17804536]
- Campbell DB, Sutcliffe JS, Ebert PJ, Militerni R, Bravaccio C, Trillo S, Elia M, Schneider C, Melmed R, Sacco R, Persico AM, Levitt P. A genetic variant that disrupts MET transcription is associated with autism. *Proc Natl Acad Sci USA* 2006;103:16834–16839. [PubMed: 17053076]
- Carpenter DO. Polychlorinated biphenyls (PCBs): routes of exposure and effects on human health. *Rev Environ Health* 2006;21:1–23. [PubMed: 16700427]
- Chen L, Durkin KA, Casida JE. Structural model for gamma-aminobutyric acid receptor noncompetitive antagonist binding: widely diverse structures fit the same site. *Proc Natl Acad Sci U S A* 2006;103:5185–5190. [PubMed: 16537435]
- Cheon KA, Ryu YH, Kim YK, Namkoong K, Kim CH, Lee JD. Dopamine transporter density in the basal ganglia assessed with [¹²³I]IPT SPET in children with attention deficit hyperactivity disorder. *Eur J Nucl Med Mol Imaging* 2003;30:306–11. [PubMed: 12552351]
- Cohly HH, Panja A. Immunological findings in autism. *Int Rev Neurobiol* 2005;71:317–341. [PubMed: 16512356]
- Cole LM, Casida JE. Polychlorocycloalkane insecticide-induced convulsions in mice in relation to disruption of the GABA-regulated chloride ionophore. *Life Sci* 1986;39:1855–1862. [PubMed: 2430161]
- Connolly AM, Chez M, Streif EM, Keeling RM, Golumbek PT, Kwon JM, Riviello JJ, Robinson RG, Neuman RJ, Deuel RM. Brain-derived neurotrophic factor and autoantibodies to neural antigens in sera of children with autistic spectrum disorders, Landau-Kleffner syndrome, and epilepsy. *Biol Psych* 2006;59:354–363.
- Connolly AM, Chez MG, Pestronk A, Arnold ST, Mehta S, Deuel RK. Serum autoantibodies to brain in Landau-Kleffner variant, autism, and other neurologic disorders. *J Pediatr* 1999;134:607–613. [PubMed: 10228297]
- Crofton KM, Kodavanti PR, Derr-Yellin EC, Casey AC, Kehn LS. PCBs, thyroid hormones, and ototoxicity in rats: cross-fostering experiments demonstrate the impact of postnatal lactation exposure. *Toxicol Sci* 2000;57:131–140. [PubMed: 10966519]
- Culiat CT, Stubbs LJ, Montgomery CS, Russell LB, Rinchik EM. Phenotypic consequences of deletion of the gamma 3, alpha 5, or beta 3 subunit of the type A gamma-aminobutyric acid receptor in mice. *Proc Natl Acad Sci U S A* 1994;91:2815–8. [PubMed: 8146195]
- Cunningham C, Champion S, Teeling J, Felton L, Perry VH. The sickness behaviour and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C). *Brain Behav Immun* 2007;21:490–502. [PubMed: 17321719]
- Dale YR, Eltom SE. Calpain mediates the dioxin-induced activation and down-regulation of the aryl hydrocarbon receptor. *Mol Pharmacol* 2006;70:1481–1487. [PubMed: 16891617]
- Dalton P, Deacon R, Blamire A, Pike M, McKinlay I, Stein J, Styles P, Vincent A. Maternal neuronal antibodies associated with autism and a language disorder. *Ann Neurol* 2003;53:533–537. [PubMed: 12666123]
- D'Amelio M, Ricci I, Sacco R, Liu X, D'Agruma L, Muscarella LA, Guarnieri V, Militerni R, Bravaccio C, Elia M, Schneider C, Melmed R, Trillo S, Pascucci T, Puglisi-Allegra S, Reichelt KL, Macciardi F, Holden JJ, Persico AM. Paraoxonase gene variants are associated with autism in North America, but not in Italy: possible regional specificity in gene-environment interactions. *Mol Psychiatry* 2005;10:1006–1016. [PubMed: 16027737]

- Davids E, Zhang K, Tarazi FI, Baldessarini RJ. Animal models of attention-deficit hyperactivity disorder. *Brain Res Rev* 2003;42:1–21. [PubMed: 12668288]
- DeCaprio AP, Johnson GW, Tarbell AM, Carpenter DO, Chiarenzelli JR, Morse GS, Santiago-Rivera AL, Schymura MJ. Polychlorinated biphenyl (PCB) exposure assessment by multivariate statistical analysis of serum congener profiles in an adult Native American population. *Environ Res* 2005;98:284–302. [PubMed: 15910784]
- DeLorey TM, Handforth A, Anagnostaras SG, Homanics GE, Minassian BA, Asaturian A, Fanselow MS, Delgado-Escueta A, Ellison GD, Olsen RW. Mice lacking the beta3 subunit of the GABAA receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. *J Neurosci* 1998;18:8505–14. [PubMed: 9763493]
- DiCicco-Bloom E, Lord C, Zwaigenbaum L, Courchesne E, Dager SR, Schmitz C, Schultz RT, Crawley J, Young LJ. The developmental neurobiology of autism spectrum disorder. *J Neurosci* 2006;26:6897–6906. [PubMed: 16807320]
- Dulce Madeira M, Pereira A, Cadete-Leite A, Paula-Barbosa MM. Estimates of volumes and pyramidal cell numbers in the prelimbic subarea of the prefrontal cortex in experimental hypothyroid rats. *J Anat* 1990;171:41–56. [PubMed: 2081709]
- Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004;429:457–63. [PubMed: 15164071]
- Evans IM, Sinha AK, Pickard MR, Edwards PR, Leonard AJ. Maternal hypothyroxinemia disrupts neurotransmitter metabolic enzymes in developing brain. *Journal of Endocrinology* 1999;161:273–79. [PubMed: 10320825]
- Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, Morga N, Jewell NP. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Perspect* 2007;115:792–798. [PubMed: 17520070]
- Fatemi, SH. *Neuropsychiatric Disorders and Infection*. Taylor & Francis; London:
- Folstein SE, Rosen-Sheidley B. Genetics of autism: complex aetiology for a heterogeneous disorder. *Nat Rev Genet* 2001;2:943–955. [PubMed: 11733747]
- Fortier ME, Kent S, Ashdown H, Poole S, Boksa P, Luheshi GN. The viral mimic, polyinosinic:polycytidylic acid, induces fever in rats via an interleukin-1-dependent mechanism. *Am J Physiol Regul Integr Comp Physiol* 2004;287:R759–766. [PubMed: 15205185]
- Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suner D, Cigudosa JC, Urioste M, Benitez J, et al. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci U S A* 2005;102:10604–9. [PubMed: 16009939]
- Fiumara A, Sciotto A, Barone R, D'Asero G, Munda S, Parano E, Pavone L. Peripheral lymphocyte subsets and other immune aspects in Rett syndrome. *Pediatric Neurology* 1999;21:619–621. [PubMed: 10513687]
- Gabriel JM, Higgins MJ, Gebuhr TC, Shows TB, Saitoh S, Nicholls RD. A model system to study genomic imprinting of human genes. *Proc Natl Acad Sci U S A* 1998;95:14857–62. [PubMed: 9843980]
- Gafni J, Wong PW, Pessah IN. Non-coplanar 2,2',3,5',6-pentachlorobiphenyl (PCB 95) amplifies ionotropic glutamate receptor signaling in embryonic cerebellar granule neurons by a mechanism involving ryanodine receptors. *Toxicol Sci* 2004;77:72–82. [PubMed: 14600284]
- Gainetdinov RR, Caron MG. Monoamine transporters: from genes to behavior. *Annu Rev Pharmacol Toxicol* 2003;43:261–84. [PubMed: 12359863]
- Garty BZ, Ludomirsky A, Danon YL, Peter JB, Douglas SD. Placental transfer of immunoglobulin G subclasses. *Clin Diagn Lab Immunol* 1994;1:667–669. [PubMed: 8556518]
- Geschwind DH, Levitt P. Autism spectrum disorders: developmental disconnection syndromes. *Curr Opin Neurobiol* 2007;17:103–111. [PubMed: 17275283]
- Gilmore JH, Jarskog LF. Exposure to infection and brain development: cytokines in the pathogenesis of schizophrenia. *Schizophr Res* 1997;24:365–367. [PubMed: 9134598]
- Gilmore JH, Jarskog LF, Vadlamudi S. Maternal poly I:C exposure during pregnancy regulates TNF alpha, BDNF, and NGF expression in neonatal brain and the maternal-fetal unit of the rat. *J Neuroimmunol* 2005;159:106–112. [PubMed: 15652408]
- Goldman LS, Genel M, Bezman RJ, Slanetz PJ. Diagnosis and treatment of attention-deficit/hyperactivity disorder in children and adolescents. *JAMA* 1998;279:1100–1107. [PubMed: 9546570]

- Hagmar L. Polychlorinated biphenyls and thyroid status in humans: a review. *Thyroid* 2003;13:1021–1028. [PubMed: 14651786]
- Hany J, Lilienthal H, Roth-Harer A, Ostendorp G, Heinzow B, Winneke G. Behavioral effects following single and combined maternal exposure to PCB 77 (3,4,3',4'-tetrachlorobiphenyl) and PCB 47 (2,4,2',4'-tetrachlorobiphenyl) in rats. *Neurotoxicol Teratol* 1999;21:147–156. [PubMed: 10192275]
- Harris NL, Spoerri I, Schopfer JF, Nembrini C, Merky P, Massacand J, Urban JF Jr, Lamarre A, Burki K, Odermatt B, Zinkernagel RM, Macpherson AJ. Mechanisms of neonatal mucosal antibody protection. *J Immunol* 2006;177:6256–6262. [PubMed: 17056555]
- Hauser P, Zametkin AJ, Martinez P, et al. Attention deficit-hyperactivity disorder in people with generalized resistance to thyroid hormone. *N Engl J Med* 1993;328:997–1001. [PubMed: 8450877]
- Heiser P, Friedel S, Dempfle A, et al. Molecular genetic aspects of attention-deficit/hyperactivity disorder. *Neurosci Biobehav Rev* 2004;28:625–41. [PubMed: 15527867]
- Hendrich B, Bickmore W. Human diseases with underlying defects in chromatin structure and modification. *Hum Mol Genet* 2001;10:2233–42. [PubMed: 11673406]
- Hertz-Picciotto I, Croen LA, Hansen R, Jones CR, van de Water J, Pessah IN. The CHARGE study: an epidemiologic investigation of genetic and environmental factors contributing to autism. *Environ Health Perspect* 2006;114:1119–1125. [PubMed: 16835068]
- Himelstein J, Schulz KP, Newcorn JH, Halperin JM. The neurobiology of attention-deficit hyperactivity disorder. *Frontiers in Bioscience* 2000;5:461–78.
- Hitchins MP, Rickard S, Dhalla F, De Vries BB, Winter R, Pembrey ME, Malcolm S. Investigation of UBE3A and MECP2 in Angelman syndrome (AS) and patients with features of AS. *Am J Med Genet* 2004;125A:167–72.
- Hogart A, Nagarajan RP, Patzel KA, Yasui DH, Lasalle JM. 15q11-13 GABAA receptor genes are normally biallelically expressed in brain yet are subject to epigenetic dysregulation in autism-spectrum disorders. *Hum Mol Genet* 2007;16:691–703. [PubMed: 17339270]
- Homanics GE, DeLorey TM, Firestone LL, Quinlan JJ, Handforth A, Harrison NL, Krasowski MD, Rick CE, Korpi ER, Makela R, et al. Mice devoid of gamma-aminobutyrate type A receptor beta3 subunit have epilepsy, cleft palate, and hypersensitive behavior. *Proc Natl Acad Sci U S A* 1997;94:4143–8. [PubMed: 9108119]
- Horike S, Cai S, Miyano M, Cheng JF, Kohwi-Shigematsu T. Loss of silent-chromatin looping and impaired imprinting of DLX5 in Rett syndrome. *Nat Genet* 2005;37:31–40. [PubMed: 15608638]
- Howard AS, Fitzpatrick R, Pessah I, Kostyniak P, Lein PJ. Polychlorinated biphenyls induce caspase-dependent cell death in cultured embryonic rat hippocampal but not cortical neurons via activation of the ryanodine receptor. *Toxicol Appl Pharmacol* 2003;190(1):72–86. [PubMed: 12831785]
- Humphrey HE, Gardiner JC, Pandya JR, Sweeney AM, Gasior DM, McCaffrey RJ, Schantz SL. PCB congener profile in the serum of humans consuming Great Lakes fish. *Environ Health Perspect* 2000;108:167–172. [PubMed: 10656858]
- Huttenlocher PR. Dendritic and synaptic pathology in mental retardation. *Pediatr Neurol* 1991;7:79–85. [PubMed: 2059260]
- Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. *N Engl J Med* 1996;335:783–89. [PubMed: 8703183]
- Jacobson JL, Jacobson SW. Prenatal exposure to polychlorinated biphenyls and attention at school age. *J Pediatr* 2003;143:780–788. [PubMed: 14657828]
- Jagadha V, Becker LE. Dendritic pathology: an overview of Golgi studies in man. *Can J Neurol Sci* 1989;16:41–50. [PubMed: 2647251]
- Jameson RR, Seidler FJ, Slotkin TA. Nonenzymatic functions of acetylcholinesterase splice variants in the developmental neurotoxicity of organophosphates: chlorpyrifos, chlorpyrifos oxon, and diazinon. *Environ Health Perspect* 2007;115:65–70. [PubMed: 17366821]
- Jyonouchi H, Geng L, Ruby A, Zimmerman-Bier B. Dysregulated innate immune responses in young children with autism spectrum disorders: their relationship to gastrointestinal symptoms and dietary intervention. *Neuropsychobiology* 2005;51:77–85. [PubMed: 15741748]
- Kapfhammer JP. Cellular and molecular control of dendritic growth and development of cerebellar Purkinje cells. *Prog Histochem Cytochem* 2004;39:131–182. [PubMed: 15580762]

- Kenet T, Froemcke R, Schreiner C, Pessah IN, Merzenich MM. Abnormal auditory cortex development in PCB exposed rats. *Proc Natl Acad Sci USA* 2007;104:7646–51. [PubMed: 17460041]
- Kodavanti PRS. Neurotoxicity of persistent organic pollutants: possible mode(s) of action and further considerations. *Dose Response* 2005;3:273, 273–275.
- Krey JF, Dolmetsch RE. Molecular mechanisms of autism: a possible role for Ca²⁺ signaling. *Curr Opin Neurobiol* 2007;17:112–119. [PubMed: 17275285]
- Lalande M, Calciano MA. Molecular epigenetics of Angelman syndrome. *Cell Mol Life Sci* 2007;64:947–60. [PubMed: 17347796]
- Lamb JA, Moore J, Bailey A, Monaco AP. Autism: recent molecular genetic advances. *Hum Mol Genet* 2000;9:861–8. [PubMed: 10767308]
- Lawrence LJ, Casida JE. Interactions of lindane, toxaphene and cyclodienes with brain-specific t-butylbicyclophosphorothionate receptor. *Life Sci* 1984;35:171–178. [PubMed: 6204183]
- Lee M, Martin-Ruiz C, Graham A, Court J, Jaros E, Perry R, Iversen P, Bauman M, Perry E. Nicotinic receptor abnormalities in the cerebellar cortex in autism. *Brain* 2002;125:1483–1495. [PubMed: 12076999]
- Lohmann C, Finski A, Bonhoeffer T. Local calcium transients regulate the spontaneous motility of dendritic filopodia. *Nat Neurosci* 2005;8:305–312. [PubMed: 15711541]
- Lord C, Cook EH, Leventhal BL, Amaral DG. Autism spectrum disorders. *Neuron* 2000a;28:355–363. [PubMed: 11144346]
- Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, Pickles A, Rutter M. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord* 2000b;30:205–223. [PubMed: 11055457]
- Lu J, Karadshah M, Delpire E. Developmental regulation of the neuronal-specific isoform of K-Cl cotransporter KCC2 in postnatal rat brains. *J Neurobiol* 1999;39:558–568. [PubMed: 10380077]
- Lyons HR, Land MB, Gibbs TT, Farb DH. Distinct signal transduction pathways for GABA-induced GABA(A) receptor down-regulation and uncoupling in neuronal culture: a role for voltage-gated calcium channels. *J Neurochem* 2001;78:1114–1126. [PubMed: 11553685]
- Ma DQ, Whitehead PL, Menold MM, Martin ER, Ashley-Koch AE, Mei H, Ritchie MD, Delong GR, Abramson RK, Wright HH, et al. Identification of significant association and gene-gene interaction of GABA receptor subunit genes in autism. *Am J Hum Genet* 2005;77:377–88. [PubMed: 16080114]
- Madras BK, Miller GM, Fischman AJ. The dopamine transporter and attention-deficit/hyperactivity disorder. *Biol Psychiatry* 2005;57:1397–409. [PubMed: 15950014]
- Mariussen E, Fønnum F. The effect of polychlorinated biphenyls on the high affinity uptake of the neurotransmitters dopamine, serotonin, glutamate and GABA, into rat brain synaptosomes. *Toxicology* 2001;159:11–21. [PubMed: 11250051]
- Mariussen E, Fønnum F. Neurochemical targets and behavioral effects of organohalogen compounds: an update. *Crit Rev Toxicol* 2006;36:253–289. [PubMed: 16686424]
- Martin ER, Menold MM, Wolpert CM, Bass MP, Donnelly SL, Ravan SA, Zimmerman A, Gilbert JR, Vance JM, Maddox LO, et al. Analysis of linkage disequilibrium in gamma-aminobutyric acid receptor subunit genes in autistic disorder. *Am J Med Genet* 2000;96:43–8. [PubMed: 10686550]
- Martin-Ruiz CM, Lee M, Perry RH, Baumann M, Court JA, Perry EK. Molecular analysis of nicotinic receptor expression in autism. *Brain Res Mol Brain Res* 2004;123:81–90. [PubMed: 15046869]
- McGough JJ. Attention-deficit/hyperactivity disorder pharmacogenomics. *Biol Psychiatry* 2005;57:1367–73. [PubMed: 15950009]
- Meguro M, Mitsuya K, Sui H, Shigenami K, Kugoh H, Nakao M, Oshimura M. Evidence for uniparental, paternal expression of the human GABAA receptor subunit genes, using microcell-mediated chromosome transfer. *Hum Mol Genet* 1997;6:2127–33. [PubMed: 9328477]
- Menold MM, Shao Y, Wolpert CM, Donnelly SL, Raiford KL, Martin ER, Ravan SA, Abramson RK, Wright HH, Delong GR, et al. Association analysis of chromosome 15 gaba receptor subunit genes in autistic disorder. *J Neurogenet* 2001;15:245–59. [PubMed: 12092907]
- Meyer U, Feldon J, Schedlowski M, Yee BK. Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia. *Neurosci Biobehav Rev* 2005;29:913–947. [PubMed: 15964075]

- Meyer U, Feldon J, Schedlowski M, Yee BK. Immunological stress at the maternal-foetal interface: a link between neurodevelopment and adult psychopathology. *Brain Behav Immun* 2006a;20:378–388. [PubMed: 16378711]
- Meyer U, Murray PJ, Urwyler A, Yee BK, Schedlowski M, Feldon J. Adult behavioral and pharmacological dysfunctions following disruption of the fetal brain balance between pro-inflammatory and IL-10-mediated anti-inflammatory signaling. *Mol Psychiatry*. 2008;in press
- Meyer U, Nyffeler M, Engler A, Urwyler A, Schedlowski M, Knuesel I, Yee BK, Feldon J. The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology. *J Neurosci* 2006b;26:4752–4762. [PubMed: 16672647]
- Meyer U, Nyffeler M, Schwendener S, Knuesel I, Yee BK, Feldon J. Relative prenatal and postnatal maternal contributions to schizophrenia-related neurochemical dysfunction after in utero immune challenge. *Neuropsychopharmacology* 2008b;33:441–56. [PubMed: 17443130]
- Meyer U, Nyffeler M, Yee BK, Knuesel I, Feldon J. Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development. *Brain Behav Immun*. 2008;in press
- Meyer U, Schwendener S, Feldon J, Yee BK. Prenatal and postnatal maternal contributions in the infection model of schizophrenia. *Exp Brain Res* 2006c;173:243–257. [PubMed: 16552558]
- Meyer U, Yee BK, Feldon J. The neurodevelopmental impact of prenatal infections at different times of pregnancy: the earlier the worse? *The Neuroscientist* 2007;13:241–256. [PubMed: 17519367]
- Michels G, Moss SJ. GABAA receptors: properties and trafficking. *Crit Rev Biochem Mol Biol* 2007;42:3–14. [PubMed: 17364682]
- Minassian BA, DeLorey TM, Olsen RW, Philippart M, Bronstein Y, Zhang Q, Guerrini R, Van Ness P, Livet MO, Delgado-Escueta AV. Angelman syndrome: correlations between epilepsy phenotypes and genotypes. *Ann Neurol* 1998;43:485–93. [PubMed: 9546330]
- Molloy CA, Morrow AL, Meinen-Derr J, Schleifer K, Dienger K, Manning-Courtney P, Altaye M, Wills-Karp M. Elevated cytokine levels in children with autism spectrum disorder. *J Neuroimmunol* 2006;172:198–205. [PubMed: 16360218]
- Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001;19:683–765. [PubMed: 11244051]
- Muhle R, Trentacoste SV, Rapin I. The genetics of autism. *Pediatrics* 2004;113:e472–86. [PubMed: 15121991]
- Muller RA. The study of autism as a distributed disorder. *Ment Retard Dev Disabil Res Rev* 2007;13:85–95. [PubMed: 17326118]
- Murray PJ. Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. *Curr Opin Pharmacol* 2006;6:379–386. [PubMed: 16713356]
- Nicholls RD, Gottlieb W, Russell LB, Davda M, Horsthemke B, Rinchik EM. Evaluation of potential models for imprinted and nonimprinted components of human chromosome 15q11–q13 syndromes by fine-structure homology mapping in the mouse. *Proc Natl Acad Sci U S A* 1993;90:2050–4. [PubMed: 8095339]
- Nicholson JL, Altman J. Synaptogenesis in the rat cerebellum: effects of early hypo- and hyperthyroidism. *Science* 1972;176:530–532. [PubMed: 5032356]
- Park JS, Linderholm L, Charles MJ, Athanasiadou M, Petrik J, Kocan A, Drobna B, Trnovec T, Bergman A, Hertz-Picciotto I. Polychlorinated biphenyls and their hydroxylated metabolites (OH-PCBS) in pregnant women from eastern Slovakia. *Environ Health Perspect* 2007;115:20–27. [PubMed: 17366814]
- Pasca SP, Nemes B, Vlase L, Gagy CE, Dronca E, Miu AC, Dronca M. High levels of homocysteine and low serum paraoxonase 1 arylesterase activity in children with autism. *Life Sci* 2006;78:2244–2248. [PubMed: 16297937]
- Patterson PH. Maternal infection: window on neuroimmune interactions in fetal brain development and mental illness. *Curr Opin Neurobiol* 2002;12:115–118. [PubMed: 11861174]
- Patterson PH. Neuroscience Maternal effects on schizophrenia risk. *Science* 2007;318:576–577. [PubMed: 17962542]
- Peltier J, O'neill A, Schaffer DV. PI3K/Akt and CREB regulate adult neural hippocampal progenitor proliferation and differentiation. *Dev Neurobiol* 2007;67:1348–1361. [PubMed: 17638387]

- Pennington BF. Toward a new neuropsychological model of attention-deficit/hyperactivity disorder: subtypes and multiple deficits. *Biol Psychiatry* 2005;57:1221–23. [PubMed: 15949991]
- Perry EK, Lee ML, Martin-Ruiz CM, et al. Cholinergic activity in autism: abnormalities in the cerebral cortex and basal forebrain. *Am J Psychiatry* 2001;158:1058–1066. [PubMed: 11431227]
- Pessah, IN.; Wong, PW. Etiology of PCB Neurotoxicity: From Molecules to Cellular Dysfunction. In: Robertson, L.; Hansen, L., editors. *Progress In Polychlorinated Biphenyl Toxicology*. New York, NY: Academic Press; 2001. p. 179-184.
- Pessah IN, Hansen LG, Albertson TE, Garner CE, Ta TA, Do Z, Kim KH, Wong PW. Structure-activity relationship for noncoplanar polychlorinated biphenyl congeners toward the ryanodine receptor-Ca²⁺ channel complex type 1 (RyR1). *Chem Res Toxicol* 2006;19:92–101. [PubMed: 16411661]
- Pessah, IN.; Lein, PJ. Evidence for environmental susceptibility in autism. What we need to know about Gene x Environment Interactions. In: Zimmerman, AW., editor. *Autism: Current Theories and Evidence*. Humana Press; 2008.
- Philippart M. Rett and Angelman's syndromes: models of arrested development. *Pediatr Neurol* 2001;25:288–94. [PubMed: 11704397]
- Pope CN. Organophosphorus pesticides: do they all have the same mechanism of toxicity? *J Toxicol Environ Health B Crit Rev* 1999;2:161–181. [PubMed: 10230392]
- Rami A, Patel AJ, Rabie A. Thyroid hormone and development of the rat hippocampus: morphological alterations in granule and pyramidal cells. *Neuroscience* 1986;19:1217–1226. [PubMed: 3822116]
- Redmond L, Ghosh A. Regulation of dendritic development by calcium signaling. *Cell Calcium* 2005;37:411–416. [PubMed: 15820388]
- Ricceri L, Venerosi A, Capone F, Cometa MF, Lorenzini P, Fortuna S, Calamandrei G. Developmental neurotoxicity of organophosphorous pesticides: fetal and neonatal exposure to chlorpyrifos alters sex-specific behaviors at adulthood in mice. *Toxicol Sci* 2006;93:105–113. [PubMed: 16760416]
- Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. *Env Health Perspect*. 2007Epub ahead of print
- Roegge CS, Wang VC, Powers BE, Klintsova AY, Villareal S, Greenough WT, Schantz SL. Motor impairment in rats exposed to PCBs and methylmercury during early development. *Toxicol Sci* 2004;77:315–324. [PubMed: 14600290]
- Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav* 2003;2:255–267. [PubMed: 14606691]
- Ruiz-Marcos A, Cartagena-Abella P, Martinez-Galan JR, Calvo R, Morreale de Escobar G, Escobar del Rey F. Thyroxine treatment and the recovery of pyramidal cells of the cerebral cortex from changes induced by juvenile-onset hypothyroidism. *J Neurobiol* 1994;25:808–818. [PubMed: 8089658]
- Russell VA. Dopamine hypofunction possibility results from a defect in glutamate-stimulated release of dopamine in the nucleus accumbens shell of a rat model for attention deficit hyperactivity disorder - the spontaneously hypertensive rat. *Neurosci Biobehav Rev* 2003;27:671–82. [PubMed: 14624811]
- Sagvolden T, Russell VA, Aase H, Johansen EB, Farshbaf M. Rodent models of attention-deficit/hyperactivity disorder. *Biol Psychiatry* 2005;57:1239–47. [PubMed: 15949994]
- Samaco RC, Hogart A, LaSalle JM. Epigenetic overlap in autism-spectrum neurodevelopmental disorders: MECP2 deficiency causes reduced expression of UBE3A and GABRB3. *Hum Mol Genet* 2005;14:483–492. [PubMed: 15615769]
- Sammelson RE, Caboni P, Durkin KA, Casida JE. GABA receptor antagonists and insecticides: common structural features of 4-alkyl-1-phenylpyrazoles and 4-alkyl-1-phenyltrioxabicyclooctanes. *Bioorg Med Chem* 2004;12:3345–3355. [PubMed: 15158803]
- Schantz SL, Widholm JJ, Rice DC. Effects of PCB exposure on neuropsychological function in children. *Environ Health Perspect* 2003;111:357–576. [PubMed: 12611666]
- Schroer RJ, Phelan MC, Michaelis RC, Crawford EC, Skinner SA, Cuccaro M, Simensen RJ, Bishop J, Skinner C, Fender D, et al. Autism and maternally derived aberrations of chromosome 15q. *Am J Med Genet* 1998;76:327–36. [PubMed: 9545097]

- Schuh RA, Lein PJ, Beckles RA, Jett DA. Noncholinesterase mechanisms of chlorpyrifos neurotoxicity: altered phosphorylation of Ca²⁺/cAMP response element binding protein in cultured neurons. *Toxicol Appl Pharmacol* 2002;182:176–185. [PubMed: 12140181]
- Seegal RF, Brosch KO, Okoniewski RJ. Effects of in utero and lactational exposure of the laboratory rat to 2,4,2',4'- and 3,4,3',4'-tetrachlorobiphenyl on dopamine function. *Toxicol Appl Pharmacol* 1997;146:95–103. [PubMed: 9299601]
- Seegal RF. Epidemiological and laboratory evidence of PCB-induced neurotoxicity. *Crit Rev Toxicol* 1996;26:709–737. [PubMed: 8958469]
- Seegal RF, Bush B, Brosch KO. Comparison of effects of Aroclors 1016 and 1260 on nonhuman primate catecholamine function. *Toxicology* 1991;66:145–63. [PubMed: 2014516]
- Seegal RF, Okoniewski RJ, Brosch KO, Bemis JC. Polychlorinated biphenyls alter extraneuronal but not tissue dopamine concentrations in adult rat striatum: an in vivo microdialysis study. *Environ Health Perspect* 2002;110:1113–17. [PubMed: 12417482]
- Seidman LJ, Valera EM, Makris N. Structural brain imaging of attention-deficit/hyperactivity disorder. *Biol Psychiatry* 2005;57:1263–72. [PubMed: 15949998]
- Shain W, Bush B, Seegal RF. Neurotoxicity of polychlorinated biphenyls: Structure-activity relationship of individual congeners. *Toxicol Appl Pharmacol* 1991;111:33–42. [PubMed: 1949034]
- Simister NE. Placental transport of immunoglobulin G. *Vaccine* 2003;21:3365–3369. [PubMed: 12850341]
- Singh VK, Lin SX, Newell E, Nelson C. Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism. *J Biomed Sci* 2002;9:359–364. [PubMed: 12145534]
- Singh VK, Rivas WH. Prevalence of serum antibodies to caudate nucleus in autistic children. *Neurosci Lett* 2004;355:53–56. [PubMed: 14729233]
- Singh VK, Singh EA, Warren RP. Hyperserotonemia and serotonin receptor antibodies in children with autism but not mental retardation. *Biol Psych* 1997a;41:753–755.
- Singh VK, Warren R, Averett R, Ghaziuddin M. Circulating autoantibodies to neuronal and glial filament proteins in autism. *Pediatr Neurol* 1997b;17:88–90. [PubMed: 9308986]
- Singh VK, Warren RP, Odell JD, Warren WL, Cole P. Antibodies to myelin basic protein in children with autistic behavior. *Brain Behav Immun* 1993;7:97–103. [PubMed: 7682457]
- Slotkin TA, Seidler FJ, Fumagalli F. Exposure to organophosphates reduces the expression of neurotrophic factors in neonatal rat brain regions: similarities and differences in the effects of chlorpyrifos and diazinon on the fibroblast growth factor superfamily. *Environ Health Perspect* 2007;115:909–16. [PubMed: 17589599]
- Slotkin TA, MacKillop EA, Ryde IT, Tate CA, Seidler FJ. Screening for developmental neurotoxicity using PC12 cells: comparisons of organophosphates with a carbamate, an organochlorine, and divalent nickel. *Environ Health Perspect* 2007;115:93–101. [PubMed: 17366826]
- Solanto MV. Dopamine dysfunction in AD/HD: integrating clinical and basic neuroscience research. *Behav Brain Res* 2002;130:65–71. [PubMed: 11864719]
- Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R, Napolitano C, Schwartz PJ, Joseph RM, Condouris K, Tager-Flusberg H, Priori SG, Sanguinetti MC, Keating MT. Ca(V)_{1.2} calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* 2004;119:19–31. [PubMed: 15454078]
- Stachowiak MK, Fang X, Myers JM, Dunham SM, Berezney R, Maher PA, Stachowiak EK. Integrative nuclear FGFR1 signaling (INFS) as a part of a universal “feed-forward-and-gate” signaling module that controls cell growth and differentiation. *J Cell Biochem* 2003;90:662–91. [PubMed: 14587025]
- Stein MA, Weiss RE. Thyroid function tests and neurocognitive functioning in children referred for attention deficit/hyperactivity disorder. *Psychoneuroendocrinology* 2003;28:304–16. [PubMed: 12573298]
- Stein MA, Weiss RE, Refetoff S. Neurocognitive characteristics of individuals with resistance to thyroid hormone: comparisons with individuals with attention-deficit hyperactivity disorder. *Developmental and Behavioral Pediatrics* 1995;16:406–11.
- Stellman SD, Djordjevic MV, Muscat JE, Gong L, Bernstein D, Citron ML, White A, Kemeny M, Busch E, Nafziger AN. Relative abundance of organochlorine pesticides and polychlorinated biphenyls

- in adipose tissue and serum of women in Long Island, New York. *Cancer Epidemiol Biomarkers Prev* 1998;7:489–496. [PubMed: 9641493]
- Stewart P, Reihman J, Gump B, Lonky E, Darvill T, Pagano J. Response inhibition at 8 and 9 1/2 years of age in children prenatally exposed to PCBs. *Neurotoxicol Teratol* 2005;27:771–80. [PubMed: 16198536]
- Thatcher KN, Peddada S, Yasui DH, Lasalle JM. Homologous pairing of 15q11–13 imprinted domains in brain is developmentally regulated but deficient in Rett and autism samples. *Hum Mol Genet* 2005;14:785–97. [PubMed: 15689352]
- Todd RD, Hickok JM, Anderson GM, Cohen DJ. Antibrain antibodies in infantile autism. *Biol Psych* 1988;23:644–647.
- Trikalinos TA, Karvouni A, Zintzaras E, Ylisaukko-Oja T, Peltonen L, Jarvela I, Ioannidis JP. A heterogeneity-based genome search meta-analysis for autism-spectrum disorders. *Mol Psychiatry* 2005;11:29–36. [PubMed: 16189507]
- Uylings HB, van Pelt J, Parnavelas JG, Ruiz-Marcos A. Geometrical and topological characteristics in the dendritic development of cortical pyramidal and non-pyramidal neurons. *Prog Brain Res* 1994;102:109–123. [PubMed: 7800808]
- van Gent T, Heijnen CJ, Treffers PD. Autism and the immune system. *J Child Psych Allied Disc* 1997;38:337–349.
- Viggiano D, Vallone D, Sadile A. Dysfunctions in dopamine systems and ADHD: evidence from animals and modeling. *Neural Plasticity* 2004;11:97–114. [PubMed: 15303308]
- Vincent JB, Horike SI, Choufani S, Paterson AD, Roberts W, Szatmari P, Weksberg R, Fernandez B, Scherer SW. An inversion inv(4)(p12–p15.3) in autistic siblings implicates the 4p GABA receptor gene cluster. *J Med Genet* 2006;43:429–434. [PubMed: 16556609]
- Volkmar FR, Pauls D. Autism. *Lancet* 2003;362:1133–41. [PubMed: 14550703]
- Warren RP, Foster A, Margaretten NC. Reduced natural killer cell activity in autism. *J Am Acad Child Adolesc Psychiatry* 1987;26:333–335. [PubMed: 3597287]
- Warren RP, Yonk LJ, Burger RA, Cole P, Odell JD, Warren WL, White E, Singh VK. Deficiency of suppressor-inducer (CD4+CD45RA+) T cells in autism. *Immunol Invest* 1990;19:245–251. [PubMed: 2142123]
- Watanabe Y, Fujita M, Ito Y, Okada T, Kusuoka H, Nishimura T. Brain dopamine transporter in spontaneously hypertensive rats. *J Nucl Med* 1997;38:470–474. [PubMed: 9074541]
- Watson P, Black G, Ramsden S, Barrow M, Super M, Kerr B, Clayton-Smith J. Angelman syndrome phenotype associated with mutations in MECP2, a gene encoding a methyl CpG binding protein. *J Med Genet* 2001;38:224–8. [PubMed: 11283202]
- Wills S, Cabanlit M, Bennett J, Ashwood P, Amaral D, Van de Water J. Autoantibodies in autism spectrum disorders (ASD). *Ann N Y Acad Sci* 2007;1107:79–91. [PubMed: 17804535]
- Wong PW, Garcia EF, Pessah IN. Ortho-substituted PCB95 alters intracellular calcium signaling and causes cellular acidification in PC12 cells by an immunophilin-dependent mechanism. *J Neurochem* 2001;76:450–463. [PubMed: 11208908]
- Wong PW, Brackney WR, Pessah IN. Ortho-substituted polychlorinated biphenyls alter microsomal calcium transport by direct interaction with ryanodine receptors of mammalian brain. *J Biol Chem* 1997;272:15145–53. [PubMed: 9182535]
- Yasui DH, Peddada S, Bieda MC, Vallero RO, Hogart A, Nagarajan RP, Thatcher KN, Farnham PJ, Lasalle JM. Integrated epigenomic analyses of neuronal MeCP2 reveal a role for long-range interaction with active genes. *Proc Natl Acad Sci USA* 2007;104:19416–21. [PubMed: 18042715]
- Young JI, Hong EP, Castle JC, Crespo-Barreto J, Bowman AB, Rose MF, Kang D, Richman R, Johnson JM, Berget S, et al. Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. *Proc Natl Acad Sci USA* 2005;102:17551–8. [PubMed: 16251272]
- Zimanyi I, Pessah IN. Pharmacological characterization of the specific binding of [³H]ryanodine to rat brain microsomal membranes. *Brain Res* 1991;561:181–191. [PubMed: 1666327]
- Zimmerman AW, Connors SL, Matteson KJ, Lee LC, Singer HS, Castaneda JA, Pearce DA. Maternal antibrain antibodies in autism. *Brain Behav Immun* 2007;21:351–357. [PubMed: 17029701]

- Zoeller RT. Environmental chemicals impacting the thyroid: targets and consequences. *Thyroid* 2007;17:811–817. [PubMed: 17956155]
- Zoeller RT, Dowling AL, Vas AA. Developmental exposure to polychlorinated biphenyls exerts thyroid hormone-like effects on the expression of RC3/neurogranin and myelin basic protein messenger ribonucleic acids in the developing rat brain. *Endocrinology* 2000;141:181–189. [PubMed: 10614638]
- Zoghbi HY. Postnatal neurodevelopmental disorders: meeting at the synapse? *Science* 2003;302:826–30. [PubMed: 14593168]

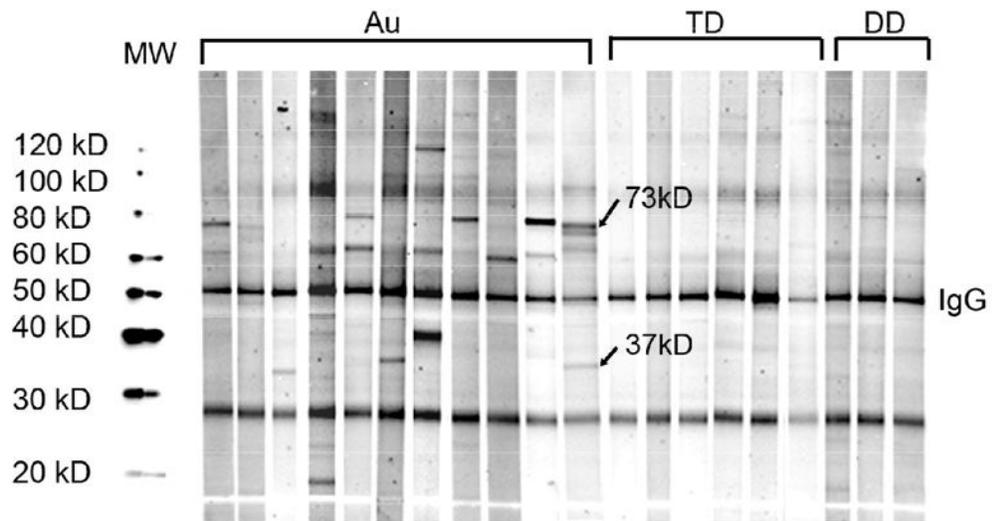


Figure 1.

Typical blot showing reactivity of maternal serum against fetal brain protein. Maternal serum diluted 1:400 was incubated with nitrocellulose strips containing human fetal brain protein and visualized using chemiluminescence and a digital imager. Bands were considered when a >2 fold densitometry measurement above background was noted.