Silent Developmental Neurotoxicity and mTOR Signaling

Suvorov A[1]

[1]Department of Environmental Health Sciences, School of Public Health and Health Sciences, University of Massachusetts, Amherst, MA 01003

Introduction

There is a high overlap in behavior traits impaired in neurodevelopmental disorders, such as autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD), and behavior traits affected by developmental exposures to persistent organohalogens in laboratory animals and humans. In recent decades, the prevalence of neurodevelopmental disorders has risen dramatically in the United States [1], the same years that the use of some organohalogens peaked as is the case for flame retardants polybrominated diphenyl ethers (PBDE). Both clinical ASD and ADHD exist as the quantitative extreme of a continuum of behavior traits [2, 3], and the epidemic of these disorders points to the possibility of a global shift in the distribution of behavior phenotypes caused by largely unknown factors, with developmental exposure to environmental pollutants being a probable candidate. Large-scale social consequences of environmentally induced subclinical neurotoxicity are known from increased murders, violent crimes, unwed pregnancies [4], and economic loss [5] in response to lead poisoning of the general population over the 20th century. This case illustrates that even a subclinical global shift of behavior traits towards socially deficient phenotypes is a cause for concern, and calls for the urgent need to identify environmental xenobiotics that may affect social behavior and to develop understanding of affected molecular mechanisms that mediate developmental neurotoxicity. Here we summarize our studies that used different models and approaches to demonstrate the possibility that perinatal exposures to a range of ubiquitous xenobiotics can alter social behavior phenotype in rodent offspring. Additionally we discuss an mTOR pathway, as a potential target of neurodevelopmental toxicity involved into the pathogenesis of ASD [6, 7] and ADHD [8, 9].

Materials and methods

Rat exposure and behavior testing. The exposure design of the experiment with Wistar rats is described elsewhere [10, 11]. In short, pregnant Wistar dams were exposed by injection to vehicle only or 0.002, 0.02 or 0.2 mg/kg body weight 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) from gestational day (GD) 15 to postnatal day (PND) 20 every 5 days. Spontaneous locomotor activity of pups was assessed using the open field test on PND 15, 20 and 25 [10].

Rat brain transcriptomics. Frontal lobe brain samples were obtained on PND41 and used for RNA extraction and transcriptome analysis using microarray approach [11]. To analyze coordinated changes in expression of coregulated gene sets we use gene set enrichment analysis (GSEA, www.broadinstitute.org/gsea/index.jsp). This approach allows to detect subtle but coordinated changes in expression by combining measurements across multiple members of each gene set [12, 13]. GSEA was performed against five predefined gene sets from a curated C2 collection of Molecular Signatures Database (www.broadinstitute.org/gsea/msigdb/collections.jsp) to reveal a signature of altered mTOR signaling [14].

Transcriptomic signature of PBDE in other tissues. To test if the same signature was altered following PBDE exposure in transcriptomic studies in other tissues we performed a search in publicly accessible databases (GEO, www.ncbi.nlm.nih.gov/geo/ and ArrayExpress (www.ebi.ac.uk/arrayexpress/) using the following key words: PBDE, BDE, polybrominated, diphenyl ether, and flame retardant. We also ran a search in PubMed (www.ncbi.nlm.nih.gov/pubmedn) using combinations of the words PBDE, BDE, and polybrominated diphenyl ether with one of the following: gene expression, transcriptome, microarray, RNA-seq, and genomic. All selected papers were then checked for the presence of all-genome gene expression analysis and, if positive, for links to the original gene expression data. As a result of this search, we identified two studies [15, 16]. One of them was performed by our group and used the same exposure protocol as described above for rat study. Total RNA was extracted from liver samples on PND27 and gene expression analysis was done using Illumina BeadChips RatRef-12 microarrays. In another study [16] Wistar Han rats were exposed to 50 mg/kg/body weight PBDE mixture DE71, which includes primarily the tetra- through penta- PBDEs and a small component of hexa-BDE. Exposure of the dams started on gestation day 6 and continued to weaning. Offspring underwent PBDE direct dosing at the same dose as their dams from PND12 for thirteen weeks after weaning. Liver samples were collected at PND22, and on week 13. Gene expression analysis was performed using Affymetrix Rat Genome 230 2.0 Array.

Mouse exposure and behavior testing. The exposure design of the experiment with CD-1 mice is described elsewhere [17]. In short, pregnant CD-1 mice were orally exposed to vehicle only or 0.2 mg/kg body weight of one of the following compounds: BDE-47, tetrabromobisphenol-A (TBBPA) or bisphenol S (BPS) from pregnancy day 8 through postpartum day 21. Three male offspring from each litter were housed together until week 10 and subjected to Three-Chamber Sociability Test.

Results and discussion

Changes in behavior traits relevant for developmental disorders. As reported in our previous study [10] perinatal exposure to all tested doses of BDE-47 results in significant hyperactivity in rat offspring on PND15-25, with highest difference in motor activity between exposed and control animals recorded on PND20. Analysis of social interactions in 10 week old mice exposed perinatally to one of 3 substances (BDE-47, TBBPA, BPS) demonstrate decreased sociability as indicated by increased mean velocity of all exposed animals when they encountered a stranger mouse and decreased time spent with conspecifics [17]. These altered behavior traits resemble behavior abnormalities diagnostic of ASD and ADHD. ASD and ADHD prevalence has risen dramatically in children in the United States in recent decades according to the U.S. Centers for Disease Control and Prevention [1]. Among children born in 2000 one in 88 is identified being as on the autism spectrum. That corresponds to a ten-fold increase in ASD prevalence in 40 years. Rates of ADHD diagnosis increased an average of 3% per year from 1997 to 2006 and an average of 5.5% per year from 2003 to 2007. Both ASD and ADHD affect the ability of patients to engage in efficient social interactions. It was shown recently that clinical ASD exist as the quantitative extreme of a continuum [2]. There is also a continuum of behavior traits from normal to subclinical, and to clinical ADHD [3]. We hypothesize that the epidemic of ASD and ADHD is the result of a global shift in the behavioral phenotypes distribution, which may be partly caused by developmental environmental exposures to substances with endocrine disruptive properties. Given that hypothesis is true, behavior of every subject in the population may be subclinically altered in the direction towards socially deficient phenotype. The subclinical perturbation of neural development due to environmental exposures to industrial chemicals, has been described as a "silent epidemic of neurotoxicity" [18]. The deleterious role of large-scale subclinical changes in mental functions for society has been graphically illustrated in relation to lead developmental exposure of U.S. population [4, 5]. Although behavioral changes similar to symptoms of lead poisoning (deficit of attention, disruptive behavior) were reported for other environmental toxins, such as methyl mercury, PCBs, dioxins, furans, and persistent pesticides [19], no analysis of large-scale social effects was done to our knowledge for any xenobiotic except lead.

Changes in tissue transcriptome following PBDE exposure. We have found that clusters of genes involved in nerve impulse transmission, nervous system development and functioning, and core biosynthetic process were altered, including several downregulated genes of cation channels in rat offspring on PND41, following exposure to low doses of BDE-47 [11]. Given that the most significantly altered functional group of genes in this study was ribosomal genes, we hypothesized that BDE-47 toxicity may be mediated via mTOR signaling. Serine/threonine protein kinase mTOR has emerged over the last decade as a critical signaling node that links nutrient sensing to the coordinated regulation of cellular growth and metabolism [20]. In response to nutrients and growth factors, mTOR complex one (mTORC1) positively regulates ribosome biogenesis [21, 22], mitochondrial biogenesis [23, 24], and adipogenesis [22, 25]. At the same time, activated mTORC1 suppresses autophagy [26] and blocks hepatic ketogenesis. To reveal a signature of altered mTOR signaling we performed GSEA for all datasets of altered gene expression in different tissues of animals exposed to PBDE. Five gene-sets were used as indicators of mTOR signaling alteration. To address changes in ribosome biogenesis the "KEGG Ribosome" gene set was used, which includes 88 human genes of ribosomal proteins and RNA (KEGG, http://www.genome.jp/kegg/). To study the alteration of mitochondria biogenesis, we used **"**Mootha Mitochondria" gene set, which includes 447 human mitochondrial genes [13]. To analyze coordinated changes in genes participating in autophagy, we used the gene set "Reactome ER Phagosome Pathway" consisting of 61 human genes (http://www.reactome.org/). The gene set "Reactome PPARα Activates Gene Expression" from the same database was used to interrogate changes in PPARα targets, which include metabolic genes responsible for ketone body production. The gene set contains 104 human genes known to be activated by PPARα from the diversity of studies. Finally, changes in expression of PPARγ targets were analyzed using the gene set "Wang Classic Adipogenic Targets of PPARγ," which includes 26 adipogenic genes induced by PPARγ during adipogenesis in mouse 3T3-L1 preadipocytes [27]. We hypothesized that if the response to PBDE exposure in mammalian organisms is mediated by the mTOR-centered pathway, then groups of functionally related molecules, targets of the pathway, would be coregulated. Namely, we predicted that ribosomal, mitochondrial genes and genes – targets of PPARγ will be regulated in one direction, while phagosomal genes and genes –

targets of PPARα will be regulated in the opposite direction in exposed animals. The results of the GSEA analysis correspond to our prediction for all 5 genomic datasets used in this study (Table 1). Surprisingly, all gene sets in the Dunnick et al. study [16] were regulated in the opposite direction from the studies done by Suvorov and Takser [11, 15] (Table 1). Enrichment scores of the 5 datasets after developmental exposure to BDE-47 are very similar for female brain frontal lobes on PND41 and livers on PND27, indicative of common mechanisms affected in both types of tissue by BDE-47. Using measurement of mTOR activity we have demonstrated recently that mTOR activity is altered in mouse liver in response to BDE-47 exposure [14]. A growing body of studies indicate involvement of mTOR pathway in the pathogenesis of neurodevelopmental disorders. The potential ability of ubiquitous environmental pollutants to disrupt mTOR signaling together with the likelihood of the silent epidemic of neurotoxicity rises significant concern.

Table 1. GSEA enrichment of functional gene groups coregulated by mTOR-centered pathway in animals exposed to PBDE (BFL – brain frontal lobes).

Sex of animals	Tissue	PND	Normalized Enrichment Score / Nominal *p* Value					Source
			Ribosome	Mitochon-dria	Phagosome	Targets of PPARα	Targets of PPARγ	
Male	liver	22	-2.14/0.000	-1.50/0.000	2.32/0.000	1.15/0.228	-1.53/0.054	[16]
Female	liver	22	-1.98/0.000	-1.68/0.000	1.22/0.206	1.79/0.003	-1.14/0.282	[16]
Male	liver	91	-1.97/0.000	-1.69/0.000	1.25/0.170	1.80/0.006	-1.16/0.259	[16]
Male	liver	27	1.39/0.034	1.22/0.056	-1.73/0.002	-1.00/0.438	0.98/0.502	[15]
Female	BFL	41	1.40/0.033	1.22/0.039	-1.72/0.000	-1.00/0.434	0.99/0.491	[11]

References

1. US CDC: http://www.cdc.gov/ncbddd/adhd/data.html. . 2013**:**.

2. Robinson EB, Koenen KC, McCormick MC, Munir K, Hallett V, Happe F, Plomin R, Ronald A: Evidence that autistic traits show the same etiology in the general population and at the quantitative extremes (5%, 2.5%, and 1%). Arch Gen Psychiatry. 2011;68 11**:**1113-1121; doi:10.1001/archgenpsychiatry.2011.119; 10.1001/archgenpsychiatry.2011.119.

3. Hong SB, Dwyer D, Kim JW, Park EJ, Shin MS, Kim BN, Yoo HJ, Cho IH, Bhang SY, Hong YC, Pantelis C, Cho SC: Subthreshold attention-deficit/hyperactivity disorder is associated with functional impairments across domains: a comprehensive analysis in a large-scale community study. Eur Child Adolesc Psychiatry. 2013**:**; doi:10.1007/s00787-013-0501-z.

4. Nevin R: How lead exposure relates to temporal changes in IQ, violent crime, and unwed pregnancy. Environ Res. 2000;83 1**:**1-22; doi:10.1006/enrs.1999.4045.

5. Grosse SD, Matte TD, Schwartz J, Jackson RJ: Economic gains resulting from the reduction in children's exposure to lead in the United States. Environ Health Perspect. 2002;110 6**:**563-569.

6. Gkogkas CG, Khoutorsky A, Ran I, Rampakakis E, Nevarko T, Weatherill DB, Vasuta C, Yee S, Truitt M, Dallaire P, Major F, Lasko P, Ruggero D, Nader K, Lacaille JC, Sonenberg N: Autism-related deficits via dysregulated eIF4E-dependent translational control. Nature. 2013;493 7432**:**371-377; doi:10.1038/nature11628 [doi].

7. Onore C, Yang H, Van de Water J, Ashwood P: Dynamic Akt/mTOR Signaling in Children with Autism Spectrum Disorder. Front Pediatr. 2017;5**:**43; doi:10.3389/fped.2017.00043 [doi].

8. Tsetsos F, Padmanabhuni SS, Alexander J, Karagiannidis I, Tsifintaris M, Topaloudi A, Mantzaris D, Georgitsi M, Drineas P, Paschou P: Meta-Analysis of Tourette Syndrome and Attention Deficit Hyperactivity Disorder Provides Support for a Shared Genetic Basis. Front Neurosci. 2016;10**:**340; doi:10.3389/fnins.2016.00340 [doi].

9. Kilincaslan A, Kok BE, Tekturk P, Yalcinkaya C, Ozkara C, Yapici Z: Beneficial Effects of Everolimus on Autism and Attention-Deficit/Hyperactivity Disorder Symptoms in a Group of Patients with Tuberous Sclerosis Complex. J Child Adolesc Psychopharmacol. 2017;27 4**:**383-388; doi:10.1089/cap.2016.0100 [doi].

10. Suvorov A, Girard S, Lachapelle S, Abdelouahab N, Sebire G, Takser L: Perinatal exposure to low-dose BDE-47, an emergent environmental contaminant, causes hyperactivity in rat offspring. Neonatology. 2009;95 3**:**203-209; doi:10.1159/000155651 [doi].

11. Suvorov A, Takser L: Delayed Response in the Rat Frontal Lobe Transcriptome to Perinatal Exposure to the Flame Retardant BDE-47. J Appl Toxicol. 2011;31 5**:**477-83.

12. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP: Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102 43**:**15545-50; doi:0506580102 [pii] 10.1073/pnas.0506580102.

13. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, Groop LC: PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet. 2003;34 3**:**267-273; doi:10.1038/ng1180 [doi].

14. Khalil A, Parker M, Mpanga R, Cevik EC, Thorburn C, Suvorov A: Developmental Exposure to 2,2′,4,4′–Tetrabromodiphenyl Ether Induces Long-Lasting Changes in Liver Metabolism in Male Mice. J Endocr Soc. 2017;1(4)**:**323-344.

15. Suvorov A, Takser L: Global Gene Expression Analysis in the Livers of Rat Offspring Perinatally Exposed to Low Doses of 2,2',4,4'-tetrabromodiphenyl ether. Environmental Health Perspectives. 2010;118 1**:**97-102.

16. Dunnick JK, Brix A, Cunny H, Vallant M, Shockley KR: Characterization of polybrominated diphenyl ether toxicity in Wistar Han rats and use of liver microarray data for predicting disease susceptibilities. Toxicol Pathol. 2012;40 1**:**93-106; doi:10.1177/0192623311429973 [doi].

17. Kim B, Colon E, Chawla S, Vandenberg LN, Suvorov A: Endocrine Disruptors Alter Social Behaviors and Indirectly Influence Social Hierarchies *via* Changes in Body Weight. Environ Health. 2015**:**; doi:10.1186/s12940-015-0051-6.

18. Grandjean P, Landrigan PJ: Developmental neurotoxicity of industrial chemicals. Lancet. 2006;368 9553**:**2167-78.

19. Carpenter DO, Nevin R: Environmental causes of violence. Physiol Behav. 2010;99 2**:**260-268; doi:10.1016/j.physbeh.2009.09.001; 10.1016/j.physbeh.2009.09.001.

20. Dibble CC, Manning BD: Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. Nat Cell Biol. 2013;15 6**:**555-564; doi:10.1038/ncb2763 [doi].

21. Conn CS, Qian SB: mTOR signaling in protein homeostasis: less is more? Cell Cycle. 2011;10 12**:**1940-1947; doi:15858 [pii].

22. Laplante M, Sabatini DM: Regulation of mTORC1 and its impact on gene expression at a glance. J Cell Sci. 2013;126 Pt 8**:**1713-1719; doi:10.1242/jcs.125773 [doi].

23. Koyanagi M, Asahara S, Matsuda T, Hashimoto N, Shigeyama Y, Shibutani Y, Kanno A, Fuchita M, Mikami T, Hosooka T, Inoue H, Matsumoto M, Koike M, Uchiyama Y, Noda T, Seino S, Kasuga M, Kido Y: Ablation of TSC2 enhances insulin secretion by increasing the number of mitochondria through activation of mTORC1. PLoS One. 2011;6 8**:**e23238; doi:10.1371/journal.pone.0023238 [doi].

24. Shende P, Plaisance I, Morandi C, Pellieux C, Berthonneche C, Zorzato F, Krishnan J, Lerch R, Hall MN, Ruegg MA, Pedrazzini T, Brink M: Cardiac raptor ablation impairs adaptive hypertrophy, alters metabolic gene expression, and causes heart failure in mice. Circulation. 2011;123 10**:**1073-1082; doi:10.1161/CIRCULATIONAHA.110.977066 [doi].

25. Zhang HH, Huang J, Duvel K, Boback B, Wu S, Squillace RM, Wu CL, Manning BD: Insulin stimulates adipogenesis through the Akt-TSC2-mTORC1 pathway. PLoS One. 2009;4 7**:**e6189; doi:10.1371/journal.pone.0006189 [doi].

26. Martina JA, Chen Y, Gucek M, Puertollano R: MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB. Autophagy. 2012;8 6**:**903-914; doi:10.4161/auto.19653 [doi].

27. Wang H, Qiang L, Farmer SR: Identification of a domain within peroxisome proliferator-activated receptor gamma regulating expression of a group of genes containing fibroblast growth factor 21 that are selectively repressed by SIRT1 in adipocytes. Mol Cell Biol. 2008;28 1**:**188-200; doi:MCB.00992-07 [pii].