Published in final edited form as:

Neurotoxicology. 2017 March ; 59: 140–154. doi:10.1016/j.neuro.2015.12.014.

Developmental Neurotoxicity of Inhaled Ambient Ultrafine Particle Air Pollution: Parallels with Neuropathological and Behavioral Features of Autism and Other Neurodevelopmental Disorders

J. L. Allen^{1,*}, G. Oberdorster¹, K. Morris-Schafer¹, C. Wong¹, C. Klocke¹, M. Sobolewski¹, K. Conrad¹, M. Mayer-Proschel², and D. A. Cory-Slechta¹

¹Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY 14642

²Department of Biomedical Genetics, University of Rochester School of Medicine, Rochester, NY 14642

Abstract

Accumulating evidence from both human and animal studies show that brain is a target of air pollution. Multiple epidemiological studies have now linked components of air pollution to diagnosis of autism spectrum disorder (ASD), a linkage with plausibility based on the shared mechanisms of inflammation. Additional plausibility appears to be provided by findings from our studies in mice of exposures from postnatal day (PND) 4-7 and 10-13 (human 3rd trimester equivalent), to concentrated ambient ultrafine (UFP) particles, considered the most reactive component of air pollution, at levels consistent with high traffic areas of major U.S. cities and thus highly relevant to human exposures. These exposures, occurring during a period of marked neuro- and gliogenesis, unexpectedly produced a pattern of developmental neurotoxicity notably similar to multiple hypothesized mechanistic underpinnings of ASD, including its greater impact in males. UFP exposures induced inflammation/microglial activation, reductions in size of the corpus callosum (CC) and associated hypomyelination, aberrant white matter development and/or structural integrity with ventriculomegaly (VM), elevated glutamate and excitatory/inhibitory imbalance, increased amygdala astrocytic activation, and repetitive and impulsive behaviors. Collectively, these findings suggest the human 3rd trimester equivalent as a period of potential vulnerability to neurodevelopmental toxicity to UFP, particularly in males, and point to the possibility that UFP air pollution exposure during periods of rapid neuro- and gliogenesis may be a risk factor not only for ASD, but also for other neurodevelopmental disorders that share features with ASD, such as schizophrenia, attention deficit disorder, and periventricular leukomalacia.

*Corresponding author at: Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

ultrafine particles; air pollution; white matter; corpus callosum; lateral ventricle; myelination; autism; schizophrenia; periventricular leukomalacia; excitatory-inhibitory imbalance

Introduction

Air Pollution Exposure

Air pollution is a complex mixture of particles, gases, trace metals and adsorbed organic contaminants. Particle sizes range from coarse (2.5-10 μ m) to fine (<2.5 μ m) to ultrafine (UFP, <100 nm or 0.1 μ m). Although not a significant component by mass, UFPs achieve orders of magnitude higher particle number concentrations and thus have more extensive surface areas that can adsorb toxic air pollutants (oxidant gases such as ozone, NO_x, organic compounds, transition metals) per unit mass. Ambient UFPs arise primarily from combustion of fossil fuels (i.e., motor vehicle traffic) [1]. Levels of up to 50% of inhaled UFP are deposited in pulmonary alveolar regions of lung from where they can traverse the alveolocapillary barrier to access pulmonary interstitium and cross endothelial cells into blood circulation and subsequently impact other organs, including heart and brain, and thereby lead to more serious health consequences, which is why UFPs are considered among the most reactive elements of air pollution [2,3]. Deposition can also occur in the nasal cavity allowing translocation to brain [4-6].

Air pollution is a worldwide environmental health problem, to which exposures begin at gestation, and are cumulative over the lifespan. While air quality standards have clearly improved in many countries, even as of 2012, the U.S. Census Bureau estimated that approximately 142 million U.S. residents live in counties where regulated levels of various air pollutants exceeded standards set by the U.S. Environmental Protection Agency (EPA). Further, ongoing urbanization trends and expanding road traffic in many areas of the world are predicted to further increase population exposures to UFP air pollution [7]. Currently, the U.S. EPA sets regulatory standards for levels of PM₁₀ and PM_{2.5}. In addition to the fact that it represents a significant technological challenge, regulation of UFPs is said to await additional evidence of their toxicity. Considered in a global context, new studies ascribe 3.3 million premature deaths per year to outdoor air pollution [8], and as of 2013, air pollution was listed as the 12th leading global risk factor for disability adjusted life year reductions [9].

Air Pollution and Brain

While the focus of air pollution research has long centered on cardiovascular and pulmonary systems, evidence now shows that air pollution also adversely impacts brain, and does so by mechanisms similar to those operative in lung, i.e., via inflammation and oxidative stress [10]. In children, various components of air pollution have been associated with impaired cognitive abilities [11,12], deficits in attention-related behaviors [13-15], reduced mental development index and IQ scores [16], symptoms of anxiety/depression [17,18], decreased nonverbal reasoning ability [19] and delayed psychomotor development [20]. Animal models confirm behavioral and CNS toxicity in response to gestational, postnatal and adult

exposures to air pollution and/or its components [10], including findings of depressive-like behaviors, impaired spatial learning and memory, reduced apical dendritic spine density and dendritic branching in hippocampus [21], reduced numbers of hippocampal neuronal GluA1 glutamate receptor subunits and altered neuronal differentiation of cortical neurons [22] and altered locomotor activity and levels of brain catecholamines [23].

Interestingly, the combined presentation of many of these specific impairments is consistent with the multifaceted nature of Autism Spectrum disorders (ASD) that has now reached a worldwide prevalence as high as 1-2% [24,25], with a clear bias towards males being more affected [25-28]. Diagnosis of ASD relies on the identification of impairments of core behavioral features like social deficits, impaired communication, and repetitive/perseverative behaviors early in life. In fact, while the etiology of this highly intractable and extremely heterogeneous neurodevelopmental disorder remains unknown, numerous epidemiological studies of populations from different states within the U.S. and different countries now report associations of ASD diagnosis with components of air pollution, including residential distance from the freeway [29], ambient levels of metals [30,31], aromatic solvents [30-32], styrene and chromium [33], gases [34-36], diesel exhaust [30,31] and particulate matter (PM_{2.5} and/or PM₁₀) [34-38]. Some report increased odds ratios for ASD diagnoses in relation to levels of PM_{2.5} [35,36,38], which includes UFPs, and/or NO₂, a surrogate for UFP [39]. The one exception to such findings was a recent study that combined data across 4 cohorts, but, unfortunately, each cohort used different measures of autistic traits, effectively limiting power and comparability [20].

Underlying reasons for an association of air pollution with ASD are not known, but several lines of evidence suggest that air pollution-induced inflammatory mechanisms during development could play a key role [40-44]. For example, markers of inflammation are elevated in amniotic fluid [45], and through life [46-48] in ASD, consistent with a chronic inflammatory state in both periphery [43,47,49,50] and brain [48,51-53]. Increased cytokine levels, a consequence of inflammation, associate with severity of ASD diagnostic features [54]. Correspondingly, multiple components of air pollution including diesel exhaust, fine as well as UFP, and toxicants adsorbed to particles, such as endotoxin (LPS), also produce inflammatory changes in brain [55-61].

Mechanistically, it has been proposed that air pollution-associated CNS pathology reflects interactive pathways that include systemic inflammation, as mediated e.g., by vagal nerve afferents [62,63], in conjunction with direct effects of particulate matter on brain tissue [10]. Perhaps not surprisingly, levels of asthma, a disease associated with air pollution-induced inflammation [64-67], are substantially higher in children with ASD, consistent with common inflammatory mechanisms in lung and brain. In a nationally representative sample of 77,951 children, asthma was 35% more common in those with ASD after controlling for age, gender, body mass index, race, brain injury, secondhand smoke and socioeconomic status [68]; further the prevalence of both ASD and asthma has been increasing [69,70].

Potential mechanistic links may also result from alterations in glutamate. Hyperglutamate function has been hypothesized to underlie ASD [71], and air pollution impacts brain glutamate function [22]. Both developing and adult microglia have functional glutamate

receptors and microglial activation can engender glutamate release [72,73]. Furthermore, glutamate release can also activate microglia, leading to a vicious cycle [74], and a potential mechanism of chronic inflammation.

This paper summarizes the results of a series of studies, including previously published findings and new data describing the impact of exposures of mice to concentrated ambient particles (CAPS) in the *ultrafine* particle (UFP) range that collectively appear to provide biological plausibility for an association between air pollution and ASD. As presented below, exposures of mice to real time, road inlet ambient concentrated (10-20×) UFP from postnatal days (PND) 4-7 and 10-13 (equivalent to human 3rd trimester [75-77], and a period of marked neuro- and gliogenesis [78]), unexpectedly produced a pattern of developmental neurotoxicity notably similar to multiple hypothesized mechanistic underpinnings of ASD and other neurodevelopmental disabilities. Our exposure paradigm is highly relevant to human exposures as UFPs, considered the most reactive component of air pollution, occurred at levels consistent with high traffic areas of major U.S. cities.

Methods

Methods presented herein are for results not previously published; methods for findings presented in prior publications are in the corresponding cited references for those findings.

Animals, Reagents, & Exposures

Male and female C57BL6/J mice 8 weeks of age were purchased from Jackson Laboratories (Bar Harbor, ME) and allowed to acclimate in the housing room for 1 week prior to breeding. Mice were co-housed for 3 days and bred monogamously, after which males were removed and pregnant dams were singly housed with litters until weaning on PND21. Pups were exposed to filtered air (Air) or concentrated ambient ultrafine particles (CAPS) using the Harvard University Concentrated Ambient Particle System (HUCAPS) fitted with a size-selective inlet and a high-volume ultrafine particle (UFPs, 100nm) concentrator (10-20×) that takes in outdoor air at 5000 liters per minute and concentrates ambient UFP, as previously described [79-82]. Exposures lasted for 4 hours per day from 0700-1200 for 4 days per week from PND4-7 and PND10-13, with exposure timing corresponding to peak vehicular traffic outside the intake valve of the HUCAPS instrumentation. A condensation particle counter (TSI 3022A) provided particle counts. Mass concentration was calculated using idealized particle density (1.5g/cm³). A Scanning Mobility Particle Sizer (SMPS) was used to determine particle size distribution and median particle diameter + GSD. Due to the presence of the particle impactor in the HUCAPS system, mice in the CAPS exposure chamber may be held at a slightly higher negative pressure compared to the Air exposed mice in an adjacent chamber. Flow of CAPS-enriched and filtered air were both maintained at 35-40% relative humidity and 77-79° F.

To preclude any effect of anesthetics on measured endpoints, brains were extracted following decapitation without sedation to assess the immediate and persistent effects of CAPS on white matter in the corpus callosum (CC), striatum (STR), and frontal cortex (FC). Brains were extracted and placed into 4% paraformaldehyde for 24 hours and then cryoprotected in 30% sucrose until they sank. Brains were stored at 4°C until they were

sectioned on a freezing microtome at 40 μm thickness. Sectioned tissue was stored in sucrose- and ethylene glycol containing cryoprotectant and kept at -20°C until immunostaining. To preclude litter-specific effects, only single pups/sex/litter were used in all of these studies. All mice used in this study were treated humanely and with regard for alleviation of suffering and approved by the University of Rochester Institutional Animal Care and Use Committee.

Particle Size Distribution and Number and Mass Concentration

Exposures in this study were to concentrated ambient UFPs ($\sim 100\text{ nm}$). As previously reported [80], mean particle diameter for these exposures was consistently below 100 nm ; mean CAPS counts across the 8 days of exposures was approximately 200,000 particles/ cm^3 , and the mean particle mass concentration was $96\text{ }\mu\text{g}/\text{m}^3$. These values are similar to values reported for expressway traffic in L. A. [83,84], and Minneapolis [85], and did not affect offspring body weights nor produce any overt toxicity.

Corpus Callosum (CC) Area Determination

The size of the CC (approximate adult-equivalent Bregma range 0.74mm - 0.2mm) was determined by tracing the area of interest in at least 3 adjacent sections of slide-mounted brain using Neuroucida (MBF, Williston, VT). Software enumerated the area of interest in μm^2 . Bregma for PND14 brains are approximate given that, to our knowledge, no atlas at that specific point of early postnatal brain development exists. Given difficulties in demarcation, all analyses of CC would also have included the cingulum, a primarily ipsilateral white matter tract lying above the CC that encircles the brain and provides communicative structure for components of the limbic system, critical to executive functions, decision-making and emotion. Dysregulation of cingulate bundle white matter development has also been linked to developmental disorders including ASD [86,87], schizophrenia [88] and ADHD [89].

Myelin Basic Protein Immunostaining & Image Analysis

To determine the extent of myelination, every 4th section (PND14) or every 6th section (PND270) was stained for myelin basic protein (MBP) to assess global development and developmental trajectory of myelination in the CC (separately examining truncus (immediately posterior to the genu), medial external capsule and lateral external capsule), striatum, and frontal cortex. Briefly, brain sections were washed of cryoprotectant and placed into primary antibody for MBP (EMD Millipore; MAB386). Tissue was then placed into biotinylated secondary antibody (Vector labs; BA-9401; 1:200 dilution) for 1 hour and the stain was visualized using 3-3'-diaminobenzidine (DAB). Immunolabeled tissue was mounted onto Superfrost Plus micro slides (VWR; Radnor, PA; 48311-703) and cover-slipped using Cytoseal 60 (Fisher Scientific; Pittsburgh, PA; 23-244257).

Briefly, at least 3 images per region of interest were captured using an Olympus BX-41 brightfield microscope prior to image analysis. Expression of MBP was analyzed using Image Pro Plus 7.0 (Mediacybernetics) using segmentation and thresholding to estimate a percent area of the region of interest that was positive for MBP expression. For CC, percent

of area myelinated was determined, whereas total relative units of myelination were measured in frontal cortex and striatum.

Statistical Analyses

Statistical analysis was carried out using JMP11 (Cary, NC) with initial between groups ANOVAs that included all experimental factors (sex, group, PN) in full factorial designs. Main effects and interactions were then pursued in post-hoc t- testing as appropriate. P values ≤ 0.05 were considered statistically significant.

Results

1. Postnatal CAPS Produces Male-Specific Microglial (IBA-1) Activation, reminiscent of inflammation and persistent microglial activation seen in ASD [90]

We previously reported persistent and male-specific IBA-1-labeling (a marker for microglia) in male but not female CAPS-treated mice, as found in anterior commissure at PND14 and hippocampus at PND55 (Fig. 1A) and in CC at PND 270 (Fig. 1B), at which point microglial numbers were approximately 350% of Air controls, even though exposures had ended at PND13 [80,81]. Microglial activation has been frequently observed in the context of ASD. For example, adult males with ASD showed increased binding of a microglial radiotracer in cerebellum, midbrain, pons, fusiform gyri and anterior cingulate and orbitofrontal cortices relative to age and IQ-matched healthy controls [91]. Post-mortem brains from individuals with ASD showed increased astrocyte- and microglial specific markers in frontal cortex [92-95]. Inflammatory markers have also been detected in blood and urine in ASD [48,96]. Early microglial activation in developing CNS [97] could be the source of altered cytokine profiles where inflammatory signals have been shown to predominate [98]. Collectively, these findings are consistent with a persisting inflammatory state.

2. Postnatal CAPS and Male-Biased Ventriculomegaly (VM) and White Matter Tract Disruption mimic aberrant white matter tract development and VM noted in ASD [99,100]

We found a number of white matter tract changes in CAPS-exposed mice that are consistent with changes seen in ASD:

Lateral Ventricle Size—Unexpectedly, a *male-specific* VM of variable severity was seen (Fig. 2) in CAPS males, but not in Air males or in any CAPS females [80]. Quantitation of VM size was determined at 3 time points, and showed VM in males to be persistent, being statistically significantly lower than Air control values out to at least PND 270 (Fig. 3) [80].

CC Size and Developmental Trajectory—Because of the suggestion of a disrupted CC (Fig 2E), CC area was quantified. Prior studies have indicated rapid postnatal growth in CC with adult-like patterns of connections by PND15 [101]. In our studies, female CC size was slightly smaller than males at PND14 (Figs. 4A and 4C), but larger at PND 270 (Fig 4B, 4D) as shown in the trajectory across time (Fig. 4E; sex by time point, $F(1,41)=57.9$, $p<0.0001$), findings consistent with previous reports of greater increases in CC size in female rats between adulthood and middle age [102] that is considered to reflect the initially larger

number and greater size of myelinated axons in males [102] and more delayed maturation to the adult pattern in females [103].

At PND14, both males and females exhibited statistically significant CAPS-induced reductions in CC size (Figs. 4A and 4C), whereas no significant differences were found at PND270 (Figs. 4B and 4D). While CAPS-treated males showed a 30% size reduction compared to 10% in CAPS-treated females at PND14 relative to Air controls (Figs 4A, 4C), statistical analysis of PND14 data confirmed only an effect of treatment ($F(1,14)=8.1$, $p=0.013$), but not a treatment by sex interaction that would statistically confirm greater magnitude effects in males. However, as can be seen from the plotted individual data, differences among males at PND14 included a broader decrement with some overlap with Air controls, while evidence for female effects based on individual data were less biologically compelling. As can be observed in Fig. 4E, there appears to be no disruption of the trajectory of CC size in females, whereas an initial blunting is clear in males.

CC Myelination—The potential for altered myelination patterns was examined using MBP staining at three different sites along the CC, i.e., the truncus, the medial external capsule (MEC) and the lateral external capsule (LEC). At PND14 (Fig. 5A and 5B), marked reductions of approximately 35% in MBP staining within the CC were seen in males at the truncus that also extended to the medial external capsule (approximately 15%), whereas no significant differences in MBP staining area were seen in females at any point along the CC (overall ANOVA: treatment by sex by region, $F(2,28)=3.39$, $p=0.048$; post hoc p values for male PND14: site by treatment, $p=0.018$; truncus, $p=0.005$; medial external capsule, $p=0.028$). At PND270 (Fig. 5C and 5D), no statistically significant treatment related effects were found in either sex despite a visual trend towards hypomyelination in CAPS-treated females.

Fig. 5E-G present time-course trajectory changes in myelination for the truncus, medial external capsule and lateral external capsule, respectively. Consistent with the early sex-specific hypomyelination in males, significant treatment by sex $\times$ time point interactions were found for truncus and medial external capsule ($F(1,42)=12.73$, $p=0.0009$ and $F(1,42)=6.62$, $p=0.0137$, respectively). No CAPS-related changes in trajectory were found in the lateral external capsule, but, consistent with observations in truncus and medial external capsule, increases in area myelination occurred between PND14 and PND270.

In contrast to the CC, striatal myelination levels of females, while significantly higher than those of males at both time points (Fig. 6A, 6B) and also when considered across time (Fig. 6C, overall ANOVA: sex by time point, $F(1,40)=4.19$, $p=0.047$), showed no effects of CAPS in either sex. Levels of MBP staining in frontal cortex were generally quite low (Figs. 7A and 7B), although increasing between PND14 and PND270 (Fig. 7C), but did not differ by sex or CAPS treatment.

Some of these changes have also been reported to occur in ASD, including reductions in area and myelination of the corpus callosum (CC), the largest white matter tract in the brain [40,104] and a tract that is critical to inter-hemispheric communication/integration for sensory, motor and higher cognitive functions [102]. The consequences of such

hypomyelination and reduced CC include under-connectivity of the two hemispheres, and increased localized connectivity. Both correlate with social and language deficits and altered hand preference/usage in ASD [105-109], an inter-hemispherically-based behavior. The particular thinning of the genu of the CC in ASD is notable, given its importance to frontal cortical connectivity, and it has been seen in conjunction with increased brain size and complexity of gyrification [110]. Despite some inconsistencies in profiles of white matter tract alterations in ASD [111], diminished CC size has been a salient manifestation [112].

The observed VM we identified in CAPS exposure has also been reported in ASD and while this is not a prototypical pathology in ASD, it is a clinical indicator of white matter damage, and often accompanies CC disruption in ASD, likely reflecting an expansion of the ventricles to fill available space [113] due to delayed development and/or cell loss. In a low for birth weight cohort, ventricular enlargement in the first week of life was associated with a 7-fold increase in ASD diagnosis at 16 and 21 years of age [114], and children with VM are found to be more likely to develop ASD [115].

3. Postnatal CAPS Persistently Elevated Glutamate Levels and Resulted in Male-Specific Excitatory-Inhibitory Imbalance, a pathology that is mirrored in ASD [116]

CAPS increased total levels of frontal cortex glutamate, glutamine and GABA levels in both sexes at various times following postnatal exposures, as we have previously reported [80-82]. In males, but not females, glutamate levels in hippocampus were also elevated, suggesting potential for greater CAPS-associated excitotoxicity. Indeed, calculated as [Glu]/[GABA] ratios, only males showed significant hippocampal excitatory/inhibitory imbalance as shown for PND14 and PND60 (Fig. 8), as GABA increases were less pronounced than those of glutamate in males. These findings are consistent with the male specificity of microglial activation (Fig. 1) and its potential to produce glutamate release [72,73] seen in ASD.

It has been reported that children and adults with ASD show altered glutamatergic balance, both in the periphery and CNS [117-122], which can correlate with altered social functioning [119]. Single nucleotide polymorphisms in glutamate transporter genes [71], and astrocyte specific dysfunctions that affect glutamate metabolism [123], have been found in genetic variants in ASD. These variants affect genes in the neurexin-neuroligin gene superfamily that are important to regulation of glutamatergic synapse formation [124]. Collectively these finding contribute to the 'hyperglutamate theory' of ASD [71]. As noted above, microglial activation/inflammation could trigger glutamate release [72,73], and glutamate release further activate microglia [74].

4. Postnatal CAPS Produces Astrocytic Activation in Amygdala consistent with the aberrant amygdala theory of ASD [125-127]

We have identified significant increases in the astrocyte specific marker GFAP in amygdala of CAPS-treated males at PND14 (Fig. 9). This adds yet another brain region impacted by UFPs that is considered key to aberrant behaviors involved in ASD [125]. Data from females and later time points are not yet examined, so the extent to which this effect is sex-differential is not yet known.

ASD brains also show altered amygdala volume, marked by early enlargement and normal or reduced volumes in adulthood, that can be linked to social abnormalities in ASD [128,129]. A recent report cited fewer amygdala neurons in some males diagnosed with ASD [94].

5. Postnatal CAPS is associated with Impulsive-Like Behavior that has also been reported in ASD

where diagnosis is predicated on primary behavioral characteristics that include social deficits, impaired communication, and repetition/perseveration [24]. Executive functions including set shifting can also be impaired in ASD and are frequent co-morbid features of the disorder [110,130-135].

The unexpected observations of disrupted CC white matter development, VM and excitatory/inhibitory imbalance in response to CAPS treatment were found only after our completion of behavioral testing in our studies, and therefore explicit testing for these primary ASD behavioral domains has not yet been completed, nor were all behavioral assays that have been implemented carried out in both sexes. Consequently, the full extent to which postnatal CAPS reproduces ASD behavioral features and its sex-dependence is not yet fully delineated.

However, other behavioral dysfunctions associated with ASD have been found in response to CAPS treatment, including perseverative/impulsive behavior in CAPS males (females not yet tested) on a Fixed Ratio waiting for reward paradigm that required 25 lever press responses in an operant chamber to initiate a waiting component during which free reward deliveries occurred. However, the time between each successive free reward delivery doubled during the wait component, thus requiring increasingly longer wait times, and any lever press response during the waiting component re-set the 25 response requirement to re-start the wait component. Postnatal CAPS markedly decreased mean waiting time and increased numbers of fixed ratio resets in males, despite the added cost in terms of numbers of responses required for each reward delivery [79]. Fig. 10 presents cumulative records of an individual subject from each treatment group depicting this perseverative/impulsive behavior, behaviors consistent with ASD-like repetition and impulsivity.

Sex-dependent deficits in executive-type behavioral domains were also found after CAPS, including alterations in learning, reversal learning, and memory even out to 14 mos (final time point of behavioral observations). Both sexes showed novel object recognition deficits (considered to reflect short term memory) [81] and impairments in repeated learning (in preparation; data not shown), i.e., ability to learn new 3-response chains for reward delivery in each successive behavioral test session, but no deficits in performing a constant already learned 3 response chains, consistent with a selective learning deficit.

Discussion

Postnatal exposures of mice to ambient UFP appear to reproduce many features consistent with characteristics of ASD, including inflammation/microglial activation (Fig. 1), reduced CC size (Fig. 4), aberrant white matter development and/or structural integrity (Figs 2-5),

hypomyelination (Fig. 5) and VM (Figs 2-3), an altered ratio of glutamate and GABA, consistent with excitatory/inhibitory imbalance (Fig. 8), and alterations in GFAP expression in the amygdala (Fig. 9), many of which were either male-specific or of greater magnitude in males. While we are still in the process of completing behavioral tests that define ASD-like behavior in animal models, we found deficits in learning, short-term memory (not shown) and, in males, repetitive and impulsive-like behaviors (Fig. 10), which are part of the primary diagnostic domain of ASD.

White Matter Damage

New analyses presented here reveal UFP-induced white matter alterations at PND14 selectively in commissural tracts (CC), with effects that were of significantly greater magnitude in males, consistent with our previous observation of male-specific and persistent microglial activation and VM [80,81]. These changes included both a significantly reduced CC size, as well as male-specific hypomyelination of CC at PND 14, with marked reductions particularly in the truncus and the medial, as opposed to the lateral external capsule. CC size ultimately reached control levels later in life following postnatal CAPS in males, whereas small but non-significant reductions in myelination were suggestive in females at PND270, findings that could indicate delayed neurotoxicity in this sex and perhaps repair in males. In contrast, no changes were seen in white matter in either striatum or in frontal cortex in either sex.

The reduction of CC size and region-specific hypomyelination coupled with our prior observations of persistent microglial activation and VM [80] is markedly similar to the clinical conditions of diffuse periventricular leukomalacia or white matter injury of prematurity/neonatal white matter damage [113,136] that can be produced by fetal inflammatory or hypoxic-ischemic injury. VM is a clinical indicator of disrupted white matter development [80,81] and can be accompanied by significant cognitive deficits [113]. Based on its well-known inflammatory properties [137], UFP exposures would be expected to activate brain microglia, resulting in the release of proinflammatory cytokines [138-140] that could lead to hypomyelination via toxicity to oligodendrocytes, the myelinating cells of the brain [141]. Indeed, particular vulnerability of premyelinating oligodendrocytes to such consequences of microglial activation has been described [142,143]. Such scenarios are considered the underlying basis of disrupted white matter development associated with prematurity [136].

Our collective findings raise several important questions. For example, what underlies the preferential male vulnerability to the early reduction in CC size and hypomyelination? One hypothesis relates to the greater number of colonizing activated microglia in male brain at the time of CAPS exposure [144]. Neuro- and gliogenesis, which derive from germinal ventricular and subventricular zones next to the ventricle walls, occurs at peak levels during the postnatal period when CAPS exposures occurred [78], and continues at a constant level into young adulthood [145,146], with activated microglia actually required for neuro- and oligodendrogenesis through the release of cytokines (potentially in combinations), including IL-1 β and IL-6 [147]. However, the greater number of activated microglia, despite its requirement for neuro- and gliogenesis to proceed, could also render male brain microglia

more vulnerable to dysfunction or even cell death in response to an ongoing inflammatory insult. Females exhibit increased numbers and more activated microglia than males only at a later postnatal age (PND30) [144], and thus CAPS exposures in these studies may have preceded the period of female vulnerability.

Other questions raised by our data are what underlies the preferential vulnerability of the truncus of the CC to CAPS-induced hypomyelination, and, what accounts for the lack of vulnerability of striatal and frontal cortex (non-commissural) white matter myelination? One potential contributor to these dichotomous effects could be regional differences in timing of myelination in early development relative to the CAPS exposure period. Interestingly, as in humans [148], myelination in mouse brain occurs earliest in the caudal part of the CC (PND14) and only later in the anterior segments, i.e., truncus and genu, e.g., between PND21-28 [149]. Myelination of regions such as striatum and cortex occurs even later, with studies showing myelination levels of only 1% in frontal cortex at PND21, up to about 15% at PND90, and up to approximately 70% at PND 180. At PND21, myelination of striatum is only about 5% of the area, and increases to about 28% by PND90 [150].

Thus, it may be that the absence of white matter myelination changes in striatum and frontal cortex reflects the fact that these processes are occurring at a sufficiently later time relative to CAPS exposures and thus are spared, and/or that any ongoing inflammation levels have subsided in that period. With respect to the vulnerability of the truncus of the CC, its primary myelination occurs 1-2 weeks post CAPS exposures, while more caudal parts of CC are undergoing myelination during the period of CAPS exposure itself. This also means that the entirety of CAPS exposure (cumulative exposure) preceded the period associated with truncus (and genu) myelination, whereas a lesser 'dose' of exposure occurred during myelination of more caudal regions. Thus, there may have been insufficient inflammation during the earlier periods of myelination, whereas a more protracted/higher level of inflammation period ended only days prior to myelination of the truncus/genu. An additional consideration is that perinatal white matter injury can be accompanied by neuronal damage [101,151], including cortical subplate neurons that are critical to formation of axons for the CC, raising the possibility that these neurons were either dysfunctional or dead thus the infrastructure for myelination never developed, a hypothesis that has yet to be examined.

Hypomyelination at the truncus, i.e., just posterior to the genu, is of particular significance given that these fibers connect motor and prefrontal cortical areas, respectively, and preserve hemispheric connectivity [152-154]. White matter alterations in these regions have been associated in adolescents with a history of preterm birth, e.g., with cognitive outcomes [155] as well as with deficits in social communication [156]. In the context of cognitive outcomes, it is notable that air pollution is also associated with prematurity and reductions in birth weight [157,158].

Air Pollution as a Broader Risk Factor: ASD Shares Features with Other Neurodevelopmental Disorders

Different components of the observed profile of CAPS-induced neuropathology are actually consistent with multiple neurodevelopmental disorders, including ASD [40,99,100,104], schizophrenia [159], and attention deficit disorder [160-167] among others, all of which also

show a higher prevalence in males, consistent with the male dominance of outcomes observed here. ASD and schizophrenia, for example, particularly childhood onset schizophrenia, are frequently considered components of a shared spectrum [168-171]. Prior to 1971 these disorders were generally lumped together as ‘childhood psychosis’, with ASD considered an early manifestation of schizophrenia. They can occur as co-morbidities, and behaviorally, both include deficits in social interactions, cognition, executive functions, emotional processing and sensorimotor gating. Both can involve auditory and visual hallucinations [170,172], abnormalities of serotonergic, dopaminergic and glutamatergic systems [173-175] and shared genetic causes [176,177]. Schizophrenia and ASD also share neuropathological features, including VM [178-180], a clinical indicator of white matter alterations that are crucial to brain interhemispheric connectivity.

Adverse pregnancy outcomes, specifically preterm birth and reduced birth weight are often associated with brain white matter alterations [181,182], as seen in conditions such as periventricular leukomalacia [183], that have been linked to inflammatory mechanisms [184-186], and can lead to cognitive impairments. In up to 50% of extremely and very preterm infants, alterations in brain white matter constitute the most important basis of axonal/neuronal damage associated with adverse neurocognitive and motor development [187]. As noted above, links between prematurity and reduced birth weight with air pollution are increasingly reported [157,158]. Alterations in brain white matter are also observed in attention deficit hyperactivity disorder [164]. Disconnectivity, i.e., functional and/or anatomical damage to white matter axons in communicating pathways found in ASD and schizophrenia [107-109,188], is critical to language, social behavior and some cognitive functions. VM associated with such deficits persists after birth [189] and is more prevalent in males [190]. Prenatal VM is predictive of time to first-episode psychosis, and schizophrenia diagnosis [179,190-193].

Animal models yielding a similar neuropathological profile to that produced by CAPS treatment can result from exposures to hypoxia, ischemia, maternal-fetal infection and lipopolysaccharide (endotoxin) administration [102]. Frequently observed in such pathology is an early necrosis in the subventricular zone adjacent to the lateral ventricle with depletion of stem cells, followed by later proliferation, as also observed in conditions such as periventricular leukomalacia [183]. Numerous studies now demonstrate the role of reactive microglia, with a particular vulnerability of premyelinating oligodendroglia, to such insults [113,187].

Conclusions

The findings reported here have two major implications: First, the data highlight the human 3rd trimester equivalent [75,77] as a period of potential vulnerability to neurodevelopmental toxicity to ambient UFP, particularly in males, adding to a growing body of evidence that brain is a target of air pollution, and point to the possibility that UFP exposure during periods of rapid neuro- and gliogenesis may be a risk factor for neurodevelopmental disorders. The most prominent period of myelination in the mouse begins at approximately PND10 [149,194,195] and thus is encompassed by our CAPS exposures. One cited limitation of rodent models of inhaled air pollution has been that rodents, unlike humans, are

obligate nose-breathers and thus dosing to mouse results in higher exposures relative to humans. However, this may be a minimal or negligent concern according to a recent study based on computational modeling using an anatomically accurate model of the human nasal cavity that found that the olfactory dose per unit surface area is actually estimated to be higher in humans for 1-7 nm particles based on the larger inhalation rates of humans [196]. The latter is of particular note as emphasized in a recent editorial [197], given the presence in fly ash and car exhaust of a significant fraction of particles below 10 nm, often overlooked because of measurement difficulty [198].

Second, ASD phenotypic expression and severity shows broad variability. If a risk factor for ASD, the variability of air pollution levels and composition across seasons/climate/geography could assist in contributing to the heterogeneity of the phenotype, which could be further compounded by the developmental timing of the exposure. Current thinking posits that the multifactorial genetic and environmental risks for ASD superimposed on different periods of brain development, could ostensibly underlie this variability of the phenotype. Air pollution, as one such risk factor, could pose a greater threat in more highly polluted regions and/or in areas where it includes more toxic elements. Inconsistencies across ASD studies could in part reflect the convergence of different sets of risk factors occurring in geographically different cohorts and significant differences in UFP exposures could also occur within even cohorts.

Acknowledgments

Supported by T32 ES007026 (PI: B.P. Lawrence), P30 ES001247 (PI: D.A. Cory-Slechta), K12 ES019852 (PI: D.A. Cory-Slechta), and R21 ES019105 (PI: D.A. Cory-Slechta).

References

1. Lippmann M, Chen LC, Gordon T, Ito K, Thurston GD. National Particle Component Toxicity (NPACT) Initiative: integrated epidemiologic and toxicologic studies of the health effects of particulate matter components. Research Report/Health Effects Institute. 2013; (177):5–13.
2. Oberdorster G, Ferin J, Lehnert BE. Correlation between particle size, in vivo particle persistence, and lung injury. Environmental Health Perspectives. 1994; 102 Suppl 5:173–9. [PubMed: 7882925]
3. Brown DM, Wilson MR, MacNee W, Stone V, Donaldson K. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicology and Applied Pharmacology. 2001; 175(3):191–9. [PubMed: 11559017]
4. Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, Potter R, Maynard A, Ito Y, Finkelstein J, Oberdorster G. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect. 2006; 114(8):1172–8. [PubMed: 16882521]
5. Hunter DD, Dey RD. Identification and neuropeptide content of trigeminal neurons innervating the rat nasal epithelium. Neuroscience. 1998; 83(2):591–9. [PubMed: 9460765]
6. Lewis J, Bench G, Myers O, Tinner B, Staines W, Barr E, Divine KK, Barrington W, Karlsson J. Trigeminal uptake and clearance of inhaled manganese chloride in rats and mice. Neurotoxicology. 2005; 26(1):113–23. [PubMed: 15527879]
7. Kumar P, Morawska L, Birmili W, Paasonen P, Hu M, Kulmala M, Harrison RM, Norford L, Britter R. Ultrafine particles in cities. Environ Int. 2014; 66:1–10. [PubMed: 24503484]
8. Lelieveld J, Evans JS, Fnais M, Giannadaki D, Pozzer A. The contribution of outdoor air pollution sources to premature mortality on a global scale. Nature. 2015; 525(7569):367–71. [PubMed: 26381985]

9. Forouzanfar MH, Alexander L, Anderson HR, Bachman VF, Biryukov S, Brauer M, Burnett R, Casey D, Coates MM, Cohen A, Delwiche K, Estep K, Frostad JJ, Kc A, Kyu HH, Moradi-Lakeh M, Ng M, Slepak EL, Thomas BA, Wagner J, Aasvang GM, Abbafati C, Ozgoren AA, Abd-Allah F, Abera SF, Aboyans V, Abraham B, Abraham JP, Abubakar I, Abu-Rmeileh NM, Aburto TC, Achoki T, Adelekan A, Adofo K, Adou AK, Adsuar JC, Afshin A, Agardh EE, Al Khabouri MJ, Al Lami FH, Alam SS, Alasfoor D, Albittar MI, Alegretti MA, Aleman AV, Alemu ZA, Alfonso-Cristancho R, Alhabib S, Ali R, Ali MK, Alla F, Allebeck P, Allen PJ, Alsharif U, Alvarez E, Alvis-Guzman N, Amankwaa AA, Amare AT, Ameh EA, Ameli O, Amini H, Ammar W, Anderson BO, Antonio CA, Anwari P, Cunningham SA, Arnlov J, Arsenijevic VS, Artaman A, Asghar RJ, Assadi R, Atkins LS, Atkinson C, Avila MA, Awuah B, Badawi A, Bahit MC, Bakfalouni T, Balakrishnan K, Balalla S, Balu RK, Banerjee A, Barber RM, Barker-Collo SL, Barquera S, Barregard L, Barrero LH, Barrientos-Gutierrez T, Basto-Abreu AC, Basu A, Basu S, Basulaiman MO, Ruvalcaba CB, Beardsley J, Bedi N, Bekele T, Bell ML, Benjet C, Bennett DA, Benzian H, Bernabe E, Beyene TJ, Bhala N, Bhalla A, Bhutta ZA, Bikbov B, Abdulhak AA, Blore JD, Blyth FM, Bohensky MA, Basara BB, Borges G, Bornstein NM, Bose D, Boufous S, Bourne RR, Brainin M, Brazinova A, Breitborde NJ, Brenner H, Briggs AD, Broday DM, Brooks PM, Bruce NG, Brugha TS, Brunekreef B, Buchbinder R, Bui LN, Bukhman G, Bulloch AG, Burch M, Burney PG, Campos-Nonato IR, Campuzano JC, Cantoral AJ, Caravanas J, Cardenas R, Cardis E, Carpenter DO, Caso V, Castaneda-Orjuela CA, Castro RE, Catala-Lopez F, Cavalleri F, Cavlin A, Chadha VK, Chang JC, Charlson FJ, Chen H, Chen W, Chen Z, Chiang PP, Chimed-Ochir O, Chowdhury R, Christophi CA, Chuang TW, Chugh SS, Cirillo M, Classen TK, Colistro V, Colomar M, Colquhoun SM, Contreras AG, Cooper C, Cooperrider K, Cooper LT, Coresh J, Courville KJ, Criqui MH, Cuevas-Nasu L, Damsere-Derry J, Danawi H, Dandona L, Dandona R, Dargan PI, Davis A, Davitoiu DV, Dayama A, de Castro EF, De la Cruz-Gongora V, De Leo D, de Lima G, Degenhardt L, Del Pozo-Cruz B, Dellavalle RP, Deribe K, Derrett S, Jarlais DC, Dessalegn M, deVeber GA, Devries KM, Dharmaratne SD, Dherani MK, Dicker D, Ding EL, Dokova K, Dorsey ER, Driscoll TR, Duan L, Durrani AM, Ebel BE, Ellenbogen RG, Elshrek YM, Endres M, Ermakov SP, Erskine HE, Eshrati B, Esteghamati A, Fahimi S, Faraon EJ, Farzadfar F, Fay DF, Feigin VL, Feigl AB, Fereshtehnejad SM, Ferrari AJ, Ferri CP, Flaxman AD, Fleming TD, Foigt N, Foreman KJ, Paleo UF, Franklin RC, Gabbe B, Gaffikin L, Gakidou E, Gamkrelidze A, Gankpe FG, Gansevoort RT, Garcia-Guerra FA, Gasana E, Geleijnse JM, Gessner BD, Gething P, Gibney KB, Gillum RF, Ginawi IA, Giroud M, Giussani G, Goenka S, Goginashvili K, Dantes HG, Gona P, de Cosio TG, Gonzalez-Castell D, Gotay CC, Goto A, Gouda HN, Guerrant RL, Gugnani HC, Guillemin F, Gunnell D, Gupta R, Gupta R, Gutierrez RA, Hafezi-Nejad N, Hagan H, Hagstromer M, Halasa YA, Hamadeh RR, Hammami M, Hankey GJ, Hao Y, Harb HL, Haregu TN, Haro JM, Havmoeller R, Hay SI, Hedayati MT, Heredia-Pi IB, Hernandez L, Heuton KR, Heydarpour P, Hajar M, Hoek HW, Hoffman HJ, Hornberger JC, Hosgood HD, Hoy DG, Hsairi M, Hu G, Hu H, Huang C, Huang JJ, Hubbell BJ, Huiart L, Hussein A, Iannarone ML, Iburg KM, Idrisov BT, Ikeda N, Innos K, Inoue M, Islami F, Ismayilova S, Jacobsen KH, Jansen HA, Jarvis DL, Jassal SK, Jauregui A, Jayaraman S, Jeemon P, Jensen PN, Jha V, Jiang F, Jiang G, Jiang Y, Jonas JB, Juel K, Kan H, Roseline SS, Karam NE, Karch A, Karema CK, Karthikeyan G, Kaul A, Kawakami N, Kazi DS, Kemp AH, Kengne AP, Keren A, Khader YS, Khalifa SE, Khan EA, Khang YH, Khatibzadeh S, Khonelidze I, Kieling C, Kim D, Kim S, Kim Y, Kimokoti RW, Kinfa Y, King JM, Kissela BM, Kivipelto M, Knibbs LD, Knudsen AK, Kokubo Y, Kose MR, Kosen S, Kraemer A, Kravchenko M, Krishnaswami S, Kromhout H, Ku T, Defo BK, Bicer BK, Kuipers EJ, Kulkarni C, Kulkarni VS, Kumar GA, Kwan GF, Lai T, Balaji AL, Lalloo R, Lallukka T, Lam H, Lan Q, Lansingh VC, Larson HJ, Larsson A, Laryea DO, Lavados PM, Lawrynowicz AE, Leasher JL, Lee JT, Leigh J, Leung R, Levi M, Li Y, Li Y, Liang J, Liang X, Lim SS, Lindsay MP, Lipshultz SE, Liu S, Liu Y, Lloyd BK, Logroscino G, London SJ, Lopez N, Lortet-Tieulent J, Lotufo PA, Lozano R, Lunevicius R, Ma J, Ma S, Machado VM, MacIntyre MF, Magis-Rodriguez C, Mahdi AA, Majdan M, Malekzadeh R, Mangalam S, Mapoma CC, Marape M, Marcenes W, Margolis DJ, Margono C, Marks GB, Martin RV, Marzan MB, Mashal MT, Masiye F, Mason-Jones AJ, Matsushita K, Matzopoulos R, Mayosi BM, Mazorodze TT, McKay AC, McKee M, McLain A, Meaney PA, Medina C, Mehndiratta MM, Mejia-Rodriguez F, Mekonnen W, Melaku YA, Meltzer M, Memish ZA, Mendoza W, Mensah GA, Meretoja A, Mhimbira FA, Michal R, Miller TR, Mills EJ, Misganaw A, Mishra S, Ibrahim NM, Mohammad KA, Mokdad AH, Mola GL, Monasta L, Hernandez JC, Montico M, Moore AR, Morawska L, Mori R, Moschandreas J, Moturi WN, Mozaffarian D, Mueller UO, Mukaigawara M,

Mullany EC, Murthy KS, Naghavi M, Nahas Z, Naheed A, Naidoo KS, Naldi L, Nand D, Nangia V, Narayan KV, Nash D, Neal B, Nejjari C, Neupane SP, Newton CR, Ngalesoni FN, de Dieu Ngirabega J, Nguyen G, Nguyen NT, Nieuwenhuijsen MJ, Nisar MI, Nogueira JR, Nolla JM, Nolte S, Norheim OF, Norman RE, Norrving B, Nyakarahuka L, Oh IH, Ohkubo T, Olusanya BO, Omer SB, Opio JN, Orozco R, Pagcatipun RS Jr, Pain AW, Pandian JD, Pabello CI, Papachristou C, Park EK, Parry CD, Caicedo AJ, Patten SB, Paul VK, Pavlin BI, Pearce N, Pedraza LS, Pedroza A, Stokic LP, Pekerici A, Pereira DM, Perez-Padilla R, Perez-Ruiz F, Perico N, Perry SA, Pervaiz A, Pesudovs K, Peterson CB, Petzold M, Phillips MR, Phua HP, Plass D, Poenaru D, Polanczyk GV, Polinder S, Pond CD, Pope CA, Pope D, Popova S, Pourmalek F, Powles J, Prabhakaran D, Prasad NM, Qato DM, Quezada AD, Quistberg DA, Racape L, Rafay A, Rahimi K, Rahimi-Movaghar V, Rahman SU, Raju M, Rakovac I, Rana SM, Rao M, Razavi H, Reddy KS, Refaat AH, Rehm J, Remuzzi G, Ribeiro AL, Riccio PM, Richardson L, Riederer A, Robinson M, Roca A, Rodriguez A, Rojas-Rueda D, Romieu I, Ronfani L, Room R, Roy N, Ruhago GM, Rushton L, Sabin N, Sacco RL, Saha S, Sahathevan R, Sahraian MA, Salomon JA, Salvo D, Sampson UK, Sanabria JR, Sanchez LM, Sanchez-Pimienta TG, Sanchez-Riera L, Sandar L, Santos IS, Sapkota A, Satpathy M, Saunders JE, Sawhney M, Saylan MI, Scarborough P, Schmidt JC, Schneider IJ, Schottker B, Schwebel DC, Scott JG, Seedat S, Sepanlou SG, Serdar B, Servan-Mori EE, Shaddick G, Shahrzad, Levy TS, Shanguan S, She J, Sheikhbahaei S, Shibuya K, Shin HH, Shinohara Y, Shiri R, Shishani K, Shiue I, Sigfusdottir ID, Silberberg DH, Simard EP, Sindi S, Singh A, Singh GM, Singh JA, Skirbekk V, Sliwa K, Soljak M, Soneji S, Soreide K, Soshnikov S, Sposato LA, Sreeramareddy CT, Stapelberg NJ, Stathopoulou V, Steckling N, Stein DJ, Stein MB, Stephens N, Stockl H, Straif K, Stroumpoulis K, Sturua L, Sunguya BF, Swaminathan S, Swaroop M, Sykes BL, Tabb KM, Takahashi K, Talongwa RT, Tandon N, Tanne D, Tanner M, Tavakkoli M, Te Ao BJ, Teixeira CM, Tellez Rojo MM, Terkawi AS, Texcalac-Sangrador JL, Thackway SV, Thomson B, Thorne-Lyman AL, Thrift AG, Thurston GD, Tillmann T, Tobollik M, Tonelli M, Topouzis F, Towbin JA, Toyoshima H, Traebert J, Tran BX, Trasande L, Trillini M, Trujillo U, Dimbuene ZT, Tsilimbaris M, Tuzcu EM, Uchendu US, Ukwaja KN, Uzun SB, van de Vijver S, Van Dingenen R, van Gool CH, van Os J, Varakin YY, Vasankari TJ, Vasconcelos AM, Vavilala MS, Veerman LJ, Velasquez-Melendez G, Venketasubramanian N, Vijayakumar L, Villalpando S, Violante FS, Vlassov VV, Vollset SE, Wagner GR, Waller SG, Wallin MT, Wan X, Wang H, Wang J, Wang L, Wang W, Wang Y, Warouw TS, Watts CH, Weichenthal S, Weiderpass E, Weintraub RG, Werdecker A, Wessells KR, Westerman R, Whiteford HA, Wilkinson JD, Williams HC, Williams TN, Woldeyohannes SM, Wolfe CD, Wong JQ, Woolf AD, Wright JL, Wurtz B, Xu G, Yan LL, Yang G, Yano Y, Ye P, Yenesew M, Yentur GK, Yip P, Yonemoto N, Yoon SJ, Younis MZ, Younoussi Z, Yu C, Zaki ME, Zhao Y, Zheng Y, Zhou M, Zhu J, Zhu S, Zou X, Zunt JR, Lopez AD, Vos T, Murray CJ. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2015

10. Block ML, Calderon-Garciduenas L. Air pollution: mechanisms of neuroinflammation and CNS disease. *Trends in Neurosciences*. 2009; 32(9):506–16. [PubMed: 19716187]
11. Harris MH, Gold DR, Rifas-Shiman SL, Melly SJ, Zanobetti A, Coull BA, Schwartz JD, Gryparis A, Kloog I, Koutrakis P, Bellinger DC, White RF, Sagiv SK, Oken E. Prenatal and Childhood Traffic-Related Pollution Exposure and Childhood Cognition in the Project Viva Cohort (Massachusetts, USA). *Environ Health Perspect*. 2015; 123(10):1072–8. [PubMed: 25839914]
12. Suglia SF, Gryparis A, Wright RO, Schwartz J, Wright RJ. Association of black carbon with cognition among children in a prospective birth cohort study. *American Journal of Epidemiology*. 2008; 167(3):280–6. [PubMed: 18006900]
13. Chiu YH, Bellinger DC, Coull BA, Anderson S, Barber R, Wright RO, Wright RJ. Associations between Traffic-Related Black Carbon Exposure and Attention in Prospective Birth Cohort of Urban Children. *Environmental Health Perspectives*. 2013
14. Newman NC, Ryan P, Lemasters G, Levin L, Bernstein D, Hershey GK, Lockey JE, Villareal M, Reponen T, Grinshpun S, Sucharew H, Dietrich KN. Traffic-related air pollution exposure in the first year of life and behavioral scores at 7 years of age. *Environmental Health Perspectives*. 2013; 121(6):731–6. [PubMed: 23694812]

15. Siddique S, Banerjee M, Ray MR, Lahiri T. Attention-deficit hyperactivity disorder in children chronically exposed to high level of vehicular pollution. *European Journal of Pediatrics*. 2011; 170(7):923–9. [PubMed: 21191614]
16. Perera FP, Li Z, Whyatt R, Hoepner L, Wang S, Camann D, Rauh V. Prenatal airborne polycyclic aromatic hydrocarbon exposure and child IQ at age 5years. *Pediatrics*. 2009; 124(2):e195–202. [PubMed: 19620194]
17. Perera FP, Rauh V, Whyatt RM, Tsai WY, Tang D, Diaz D, Hoepner L, Barr D, Tu YH, Camann D, Kinney P. Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner-city children. *Environmental Health Perspectives*. 2006; 114(8):1287–92. [PubMed: 16882541]
18. Perera FP, Tang D, Wang S, Vishnevsky J, Zhang B, Diaz D, Camann D, Rauh V. Prenatal polycyclic aromatic hydrocarbon (PAH) exposure and child behavior at age 6-7years. *Environmental Health Perspectives*. 2012; 120(6):921–6. [PubMed: 22440811]
19. Edwards SC, Jedrychowski W, Butscher M, Camann D, Kieltyka A, Mroz E, Flak E, Li Z, Wang S, Rauh V, Perera F. Prenatal exposure to airborne polycyclic aromatic hydrocarbons and children's intelligence at 5 years of age in a prospective cohort study in Poland. *Environmental Health Perspectives*. 2010; 118(9):1326–31. [PubMed: 20406721]
20. Guxens M, Ghassabian A, Gong T, Garcia-Esteban R, Porta D, Giorgis-Allemand L, Almqvist C, Aranbarri A, Beelen R, Badaloni C, Cesaroni G, de Nazelle A, Estarlich M, Forastiere F, Fornis J, Gehring U, Ibarluzea J, Jaddoe VW, Korek M, Lichtenstein P, Nieuwenhuijsen MJ, Rebagliato M, Slama R, Tiemeier H, Verhulst FC, Volk HE, Pershagen G, Brunekreef B, Sunyer J. Air Pollution Exposure during Pregnancy and Childhood Autistic Traits in Four European Population-Based Cohort Studies: The ESCAPE Project. *Environmental Health Perspectives*. 2015
21. Fonken LK, Xu X, Weil ZM, Chen G, Sun Q, Rajagopalan S, Nelson RJ. Air pollution impairs cognition, provokes depressive-like behaviors and alters hippocampal cytokine expression and morphology. *Mol Psychiatry*. 2011; 16(10):987–95, 973. [PubMed: 21727897]
22. Davis DA, Bortolato M, Godar SC, Sander TK, Iwata N, Pakbin P, Shih JC, Berhane K, McConnell R, Sioutas C, Finch CE, Morgan TE. Prenatal exposure to urban air nanoparticles in mice causes altered neuronal differentiation and depression-like responses. *PLoS One*. 2013; 8(5):e64128. [PubMed: 23734187]
23. Suzuki T, Oshio S, Iwata M, Saburi H, Odagiri T, Udagawa T, Sugawara I, Umezawa M, Takeda K. In utero exposure to a low concentration of diesel exhaust affects spontaneous locomotor activity and monoaminergic system in male mice. *Part Fibre Toxicol*. 2010; 7:7. [PubMed: 20331848]
24. Lai MC, Lombardo MV, Baron-Cohen S. Autism. *Lancet*. 2014; 383(9920):896–910. [PubMed: 24074734]
25. Elsabbagh M, Divan G, Koh YJ, Kim YS, Kauchali S, Marcin C, Montiel-Nava C, Patel V, Paula CS, Wang C, Yasamy MT, Fombonne E. Global prevalence of autism and other pervasive developmental disorders. *Autism Res*. 2012; 5(3):160–79. [PubMed: 22495912]
26. Frazier TW, Georgiades S, Bishop SL, Hardan AY. Behavioral and cognitive characteristics of females and males with autism in the Simons Simplex Collection. *Journal of the American Academy of Child and Adolescent Psychiatry*. 2014; 53(3):329–40 e1-3. [PubMed: 24565360]
27. Van Wijngaarden-Cremers PJ, van Eeten E, Groen WB, Van Deurzen PA, Oosterling IJ, Van der Gaag RJ. Gender and age differences in the core triad of impairments in autism spectrum disorders: a systematic review and meta-analysis. *J Autism Dev Disord*. 2014; 44(3):627–35. [PubMed: 23989936]
28. Werling DM, Geschwind DH. Sex differences in autism spectrum disorders. *Curr Opin Neurol*. 2013; 26(2):146–53. [PubMed: 23406909]
29. Volk HE, Hertz-Picciotto I, Delwiche L, Lurmann F, McConnell R. Residential proximity to freeways and autism in the CHARGE study. *Environmental Health Perspectives*. 2011; 119(6):873–7. [PubMed: 21156395]
30. Roberts AL, Lyall K, Hart JE, Laden F, Just AC, Bobb JF, Koenen KC, Ascherio A, Weisskopf MG. Perinatal air pollutant exposures and autism spectrum disorder in the children of Nurses' Health Study II participants. *Environmental Health Perspectives*. 2013

31. Windham GC, Zhang L, Gunier R, Croen LA, Grether JK. Autism spectrum disorders in relation to distribution of hazardous air pollutants in the sanfrancisco bay area. *Environmental Health Perspectives*. 2006; 114(9):1438–44. [PubMed: 16966102]
32. Kalkbrenner AE, Daniels JL, Chen JC, Poole C, Emch M, Morrissey J. Perinatal exposure to hazardous air pollutants and autism spectrum disorders at age 8. *Epidemiology*. 2010; 21(5):631–41. [PubMed: 20562626]
33. Talbott EO, Marshall LP, Rager JR, Arena VC, Sharma RK, Stacy SL. Air toxics and the risk of autism spectrum disorder: the results of a population based case-control study in southwestern Pennsylvania. *Environ Health*. 2015; 14:80. [PubMed: 26444407]
34. Becerra TA, Wilhelm M, Olsen J, Cockburn M, Ritz B. Ambient air pollution and autism in Los Angeles county, California. *Environmental Health Perspectives*. 2013; 121(3):380–6. [PubMed: 23249813]
35. Jung CR, Lin YT, Hwang BF. Air pollution and newly diagnostic autism spectrum disorders: a population-based cohort study in Taiwan. *PLoS One*. 2013; 8(9):e75510. [PubMed: 24086549]
36. Volk HE, Lurmann F, Penfold B, Hertz-Picciotto I, McConnell R. Traffic-related air pollution, particulate matter, and autism. *JAMA Psychiatry*. 2013; 70(1):71–7. [PubMed: 23404082]
37. Kalkbrenner AE, Windham GC, Serre ML, Akita Y, Wang X, Hoffman K, Thayer BP, Daniels JL. Particulate matter exposure, prenatal and postnatal windows of susceptibility, and autism spectrum disorders. *Epidemiology*. 2015; 26(1):30–42. [PubMed: 25286049]
38. Raz R, Roberts AL, Lyall K, Hart JE, Just AC, Laden F, Weisskopf MG. Autism Spectrum Disorder and Particulate Matter Air Pollution before, during, and after Pregnancy: A Nested Case-Control Analysis within the Nurses'Health Study II Cohort. *Environmental Health Perspectives*. 2014
39. Beckerman B, Jerrett M, Brook JR, Verman DK, Arain MA, Finkelstein MM. Correlation of nitrogen dioxide with other traffic pollutants near a major expressway. *Atmospheric Environment*. 2008; 42:275–290.
40. Melillo R, Leisman G. Autistic spectrum disorders as functional disconnection syndrome. *Reviews in the Neurosciences*. 2009; 20(2):111–31. [PubMed: 19774789]
41. Noriega DB, Savelkoul HF. Immune dysregulation in autism spectrum disorder. *European Journal of Pediatrics*. 2014; 173(1):33–43. [PubMed: 24297668]
42. Meldrum SJ, Strunk T, Currie A, Prescott SL, Simmer K, Whitehouse AJ. Autism spectrum disorder in children born preterm-role of exposure to perinatal inflammation. *Front Neurosci*. 2013; 7:123. [PubMed: 23885233]
43. Depino AM. Peripheral and central inflammation in autism spectrum disorders. *Molecular and Cellular Neurosciences*. 2013; 53:69–76. [PubMed: 23069728]
44. Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. *Brain, Behavior, and Immunity*. 2012; 26(3):383–92.
45. Abdallah MW, Larsen N, Grove J, Norgaard-Pedersen B, Thorsen P, Mortensen EL, Hougaard DM. Amniotic fluid inflammatory cytokines: potential markers of immunologic dysfunction in autism spectrum disorders. *World J Biol Psychiatry*. 2013; 14(7):528–38. [PubMed: 22175527]
46. Gesundheit B, Rosenzweig JP, Naor D, Lerer B, Zachor DA, Prochazka V, Melamed M, Kristt DA, Steinberg A, Shulman C, Hwang P, Koren G, Walfisch A, Passweg JR, Snowden JA, Tamouza R, Leboyer M, Farge-Bancel D, Ashwood P. Immunological and autoimmune considerations of Autism Spectrum Disorders. *Journal of Autoimmunity*. 2013; 44:1–7. [PubMed: 23867105]
47. Emanuele E, Orsi P, Boso M, Broglio D, Brondino N, Barale F, di Nemi SU, Politi P. Low-grade endotoxemia in patients with severe autism. *Neuroscience Letters*. 2010; 471(3):162–5. [PubMed: 20097267]
48. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals of Neurology*. 2005; 57(1):67–81. [PubMed: 15546155]
49. El-Ansary A, Al-Ayadhi L. Neuroinflammation in autism spectrum disorders. *J Neuroinflammation*. 2012; 9:265. [PubMed: 23231720]

50. Naik US, Gangadharan C, Abbagani K, Nagalla B, Dasari N, Manna SK. A study of nuclear transcription factor-kappa B in childhood autism. *PLoS One*. 2011; 6(5):e19488. [PubMed: 21573053]
51. Rose S, Melnyk S, Pavliv O, Bai S, Nick TG, Frye RE, James SJ. Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain. *Transl Psychiatry*. 2012; 2:e134. [PubMed: 22781167]
52. Wei H, Zou H, Sheikh AM, Malik M, Dobkin C, Brown WT, Li X. IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. *J Neuroinflammation*. 2011; 8:52. [PubMed: 21595886]
53. Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li XM, Ji L, Brown T, Malik M. Elevated immune response in the brain of autistic patients. *Journal of Neuroimmunology*. 2009; 207(1-2): 111–6. [PubMed: 19157572]
54. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. *J Neuroimmunol*. 2011; 232(1-2):196–9. [PubMed: 21095018]
55. Bolton JL, Smith SH, Huff NC, Gilmour MI, Foster WM, Auten RL, Bilbo SD. Prenatal air pollution exposure induces neuroinflammation and predisposes offspring to weight gain in adulthood in a sex-specific manner. *FASEB Journal*. 2012; 26(11):4743–54. [PubMed: 22815382]
56. Campbell A, Araujo JA, Li H, Sioutas C, Kleinman M. Particulate matter induced enhancement of inflammatory markers in the brains of apolipoprotein E knockout mice. *J Nanosci Nanotechnol*. 2009; 9(8):5099–104. [PubMed: 19928188]
57. Hartz AM, Bauer B, Block ML, Hong JS, Miller DS. Diesel exhaust particles induce oxidative stress, proinflammatory signaling, and P-glycoprotein up-regulation at the blood-brain barrier. *FASEB Journal*. 2008; 22(8):2723–33. [PubMed: 18474546]
58. Levesque S, Surace MJ, McDonald J, Block ML. Air pollution & the brain: Subchronic diesel exhaust exposure causes neuroinflammation and elevates early markers of neurodegenerative disease. *J Neuroinflammation*. 2011; 8:105. [PubMed: 21864400]
59. Levesque S, Taetzsch T, Lull ME, Kodavanti U, Stadler K, Wagner A, Johnson JA, Duke L, Kodavanti P, Surace MJ, Block ML. Diesel exhaust activates and primes microglia: air pollution, neuroinflammation, and regulation of dopaminergic neurotoxicity. *Environmental Health Perspectives*. 2011; 119(8):1149–55. [PubMed: 21561831]
60. Morgan TE, Davis DA, Iwata N, Tanner JA, Snyder D, Ning Z, Kam W, Hsu YT, Winkler JW, Chen JC, Petasis NA, Baudry M, Sioutas C, Finch CE. Glutamatergic neurons in rodent models respond to nanoscale particulate urban air pollutants in vivo and in vitro. *Environmental Health Perspectives*. 2011; 119(7):1003–9. [PubMed: 21724521]
61. van Berlo D, Albrecht C, Knaapen AM, Cassee FR, Gerlofs-Nijland ME, Kooter IM, Palomero-Gallagher N, Bidmon HJ, van Schooten FJ, Krutmann J, Schins RP. Comparative evaluation of the effects of short-term inhalation exposure to diesel engine exhaust on rat lung and brain. *Archives of Toxicology*. 2010; 84(7):553–62. [PubMed: 20467864]
62. Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C. Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol*. 2004; 16(6-7):437–45. [PubMed: 15204759]
63. Oberdorster G, Elder A, Rinderknecht A. Nanoparticles and the brain: cause for concern? *J Nanosci Nanotechnol*. 2009; 9(8):4996–5007. [PubMed: 19928180]
64. Wendt JK, Symanski E, Stock TH, Chan W, Du XL. Association of short-term increases in ambient air pollution and timing of initial asthma diagnosis among Medicaid-enrolled children in a metropolitan area. *Environmental Research*. 2014; 131:50–8. [PubMed: 24657516]
65. Evans KA, Halterman JS, Hopke PK, Fagnano M, Rich DQ. Increased ultrafine particles and carbon monoxide concentrations are associated with asthma exacerbation among urban children. *Environmental Research*. 2014; 129:11–9. [PubMed: 24528997]
66. MacIntyre EA, Carlsten C, MacNutt M, Fuertes E, Melen E, Tiesler CM, Gehring U, Kramer U, Klumper C, Kerkhof M, Chan-Yeung M, Kozyrskyj AL, Berdel D, Bauer CP, Herbarth O, Bauer M, Schaaf B, Koletzko S, Pershagen G, Brunekreef B, Heinrich J, Brauer M. Traffic, asthma and genetics: combining international birth cohort data to examine genetics as a mediator of traffic-

- related air pollution's impact on childhood asthma. *European Journal of Epidemiology*. 2013; 28(7):597–606. [PubMed: 23880893]
67. Gonzalez-Barcala FJ, Pertega S, Garnelo L, Castro TP, Sampedro M, Lastres JS, San Jose Gonzalez MA, Bamonde L, Valdes L, Carreira JM, Silvarrey AL. Truck traffic related air pollution associated with asthma symptoms in young boys: a cross-sectional study. *Public Health*. 2013; 127(3):275–81. [PubMed: 23434038]
 68. Kotey S, Ertel K, Whitcomb B. Co-occurrence of Autism and Asthma in a Nationally-Representative Sample of Children in the United States. *Journal of Autism and Developmental Disorders*. 2014
 69. Becker KG. Autism, asthma, inflammation, and the hygiene hypothesis. *Medical Hypotheses*. 2007; 69(4):731–40. [PubMed: 17412520]
 70. Becker KG, Schultz ST. Similarities in features of autism and asthma and a possible link to acetaminophen use. *Medical Hypotheses*. 2010; 74(1):7–11. [PubMed: 19748189]
 71. Jacob S, Brune CW, Badner JA, Ernstrom K, Courchesne E, Lord C, Leventhal BL, Cook EH, Kim SJ. Family-based association testing of glutamate transporter genes in autism. *Psychiatric Genetics*. 2011; 21(4):212–3. [PubMed: 21085054]
 72. Murugan M, Ling EA, Kaur C. Glutamate receptors in microglia. *CNS Neurol Disord Drug Targets*. 2013; 12(6):773–84. [PubMed: 24047523]
 73. Takeuchi H, Mizuno T, Zhang G, Wang J, Kawanokuchi J, Kuno R, Suzumura A. Neuritic beading induced by activated microglia is an early feature of neuronal dysfunction toward neuronal death by inhibition of mitochondrial respiration and axonal transport. *Journal of Biological Chemistry*. 2005; 280(11):10444–54. [PubMed: 15640150]
 74. Biber K, Neumann H, Inoue K, Boddeke HW. Neuronal 'On' and 'Off' signals control microglia. *Trends in Neurosciences*. 2007; 30(11):596–602. [PubMed: 17950926]
 75. Clancy B, Finlay BL, Darlington RB, Anand KJ. Extrapolating brain development from experimental species to humans. *Neurotoxicology*. 2007; 28(5):931–7. [PubMed: 17368774]
 76. Clancy B, Kersh B, Hyde J, Darlington RB, Anand KJ, Finlay BL. Web-based method for translating neurodevelopment from laboratory species to humans. *Neuroinformatics*. 2007; 5(1):79–94. [PubMed: 17426354]
 77. Rice D, Barone S Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect*. 2000; 108 Suppl 3:511–33. [PubMed: 10852851]
 78. Bandeira F, Lent R, Herculano-Houzel S. Changing numbers of neuronal and non-neuronal cells underlie postnatal brain growth in the rat. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106(33):14108–13. [PubMed: 19666520]
 79. Allen JL, Conrad K, Oberdorster G, Johnston CJ, Sleezer B, Cory-Slechta DA. Developmental exposure to concentrated ambient particles and preference for immediate reward in mice. *Environmental Health Perspectives*. 2013; 121(1):32–8. [PubMed: 23063827]
 80. Allen JL, Liu X, Pelkowski S, Palmer B, Conrad K, Oberdorster G, Weston D, Mayer-Proschel M, Cory-Slechta DA. Early postnatal exposure to ultrafine particulate matter air pollution: persistent ventriculomegaly, neurochemical disruption, and glial activation preferentially in male mice. *Environmental Health Perspectives*. 2014; 122(9):939–45. [PubMed: 24901756]
 81. Allen JL, Liu X, Weston D, Prince L, Oberdorster G, Finkelstein JN, Johnston CJ, Cory-Slechta DA. Developmental exposure to concentrated ambient ultrafine particulate matter air pollution in mice results in persistent and sex-dependent behavioral neurotoxicity and glial activation. *Toxicological Sciences*. 2014; 140(1):160–78. [PubMed: 24690596]
 82. Allen JL, Liu X, Weston D, Conrad K, Oberdorster G, Cory-Slechta DA. Consequences of developmental exposure to concentrated ambient ultrafine particle air pollution combined with the adult paraquat and maneb model of the Parkinson's disease phenotype in male mice. *Neurotoxicology*. 2014; 41:80–8. [PubMed: 24486957]
 83. Fruin S, Westerdahl D, Sax T, Sioutas C, Fine PM. Measurements and predictors of on-road ultrafine particle concentrations and associated pollutants in Los Angeles. *Atmospheric Environment*. 2008; 42:207–219.

84. Westerdahl D, Fruin S, Sax T, Fine PM, Sioutas C. mobile platform measurements of ultrafine particles and associated pollutant concentrations on freeways and residential streets in Los Angeles. *Atmospheric Environment*. 2005; 39:3597–3610.
85. Kittelson DB, Watts WF, Johnson JP, Remerowski ML, Ische EE, Oberdorster G, Gelein RM, Elder A, Hopke PK, Kim E, Zhao W, Zhou L, Jeong CH. On-road exposure to highway aerosols. 1. Aerosol and gas measurements. *Inhal Toxicol*. 2004; 16 Suppl 1:31–9. [PubMed: 15204791]
86. Billeci L, Calderoni S, Tosetti M, Catani M, Muratori F. White matter connectivity in children with autism spectrum disorders: a tract-based spatial statistics study. *BMC Neurol*. 2012; 12:148. [PubMed: 23194030]
87. Ikuta T, Shafritz KM, Bregman J, Peters BD, Gruner P, Malhotra AK, Szeszko PR. Abnormal cingulum bundle development in autism: a probabilistic tractography study. *Psychiatry Res*. 2014; 221(1):63–8. [PubMed: 24231056]
88. Samartzis L, Dima D, Fusar-Poli P, Kyriakopoulos M. White matter alterations in early stages of schizophrenia: a systematic review of diffusion tensor imaging studies. *J Neuroimaging*. 2014; 24(2):101–10. [PubMed: 23317110]
89. Chiang HL, Chen YJ, Lo YC, Tseng WY, Gau SS. Altered white matter tract property related to impaired focused attention, sustained attention, cognitive impulsivity and vigilance in attention-deficit/hyperactivity disorder. *J Psychiatry Neurosci*. 2015; 40(5):325–35. [PubMed: 25871496]
90. Zeidan-Chulia F, Salmina AB, Malinovskaya NA, Noda M, Verkhatsky A, Moreira JC. The glial perspective of autism spectrum disorders. *Neuroscience and Biobehavioral Reviews*. 2014; 38:160–72. [PubMed: 24300694]
91. Suzuki K, Sugihara G, Ouchi Y, Nakamura K, Futatsubashi M, Takebayashi K, Yoshihara Y, Omata K, Matsumoto K, Tsuchiya KJ, Iwata Y, Tsujii M, Sugiyama T, Mori N. Microglial activation in young adults with autism spectrum disorder. *JAMA Psychiatry*. 2013; 70(1):49–58. [PubMed: 23404112]
92. Edmonson C, Ziats MN, Rennert OM. Altered glial marker expression in autistic post-mortem prefrontal cortex and cerebellum. *Mol Autism*. 2014; 5(1):3. [PubMed: 24410870]
93. Young AM, Campbell E, Lynch S, Suckling J, Powis SJ. Aberrant NF-kappaB expression in autism spectrum condition: a mechanism for neuroinflammation. *Front Psychiatry*. 2011; 2:27. [PubMed: 21629840]
94. Morgan JT, Chana G, Pardo CA, Achim C, Semendeferi K, Buckwalter J, Courchesne E, Everall IP. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biological Psychiatry*. 2010; 68(4):368–76. [PubMed: 20674603]
95. Laurence JA, Fatemi SH. Glial fibrillary acidic protein is elevated in superior frontal, parietal and cerebellar cortices of autistic subjects. *Cerebellum*. 2005; 4(3):206–10. [PubMed: 16147953]
96. Rossignol DA, Frye RE. Evidence linking oxidative stress, mitochondrial dysfunction, and inflammation in the brain of individuals with autism. *Front Physiol*. 2014; 5:150. [PubMed: 24795645]
97. Bilimoria PM, Stevens B. Microglia function during brain development: New Insights from Animal Models. *Brain Research*. 2014
98. Masi A, Quintana DS, Glozier N, Lloyd AR, Hickie IB, Guastella AJ. Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis. *Molecular Psychiatry*. 2014
99. Aoki Y, Abe O, Nippashi Y, Yamasue H. Comparison of white matter integrity between autism spectrum disorder subjects and typically developing individuals: a meta-analysis of diffusion tensor imaging tractography studies. *Mol Autism*. 2013; 4(1):25. [PubMed: 23876131]
100. Keller TA, Kana RK, Just MA. A developmental study of the structural integrity of white matter in autism. *Neuroreport*. 2007; 18(1):23–7. [PubMed: 17259855]
101. Wahlsten D. Growth of the mouse corpus callosum. *Brain Res*. 1984; 317(1):59–67. [PubMed: 6467032]
102. Yates MA, Juraska JM. Increases in size and myelination of the rat corpus callosum during adulthood are maintained into old age. *Brain Res*. 2007; 1142:13–8. [PubMed: 17300760]
103. Kim JH, Juraska JM. Sex differences in the development of axon number in the splenium of the rat corpus callosum from postnatal day 15 through 60. *Brain Research. Developmental Brain Research*. 1997; 102(1):77–85. [PubMed: 9298236]

104. Barnea-Goraly N, Kwon H, Menon V, Eliez S, Lotspeich L, Reiss AL. White matter structure in autism: preliminary evidence from diffusion tensor imaging. *Biological Psychiatry*. 2004; 55(3): 323–6. [PubMed: 14744477]
105. Floris DL, Chura LR, Holt RJ, Suckling J, Bullmore ET, Baron-Cohen S, Spencer MD. Psychological correlates of handedness and corpus callosum asymmetry in autism: the left hemisphere dysfunction theory revisited. *Journal of Autism and Developmental Disorders*. 2013; 43(8):1758–72. [PubMed: 23179344]
106. Forrester GS, Pegler R, Thomas MS, Mareschal D. Handedness as a marker of cerebral lateralization in children with and without autism. *Behavioural Brain Research*. 2014; 268:14–21. [PubMed: 24704491]
107. Geschwind DH, Levitt P. Autism spectrum disorders: developmental disconnection syndromes. *Current Opinion in Neurobiology*. 2007; 17(1):103–11. [PubMed: 17275283]
108. Kana RK, Libero LE, Moore MS. Disrupted cortical connectivity theory as an explanatory model for autism spectrum disorders. *Phys Life Rev*. 2011; 8(4):410–37. [PubMed: 22018722]
109. Wass S. Distortions and disconnections: disrupted brain connectivity in autism. *Brain and Cognition*. 2011; 75(1):18–28. [PubMed: 21055864]
110. Vidal CN, Nicolson R, DeVito TJ, Hayashi KM, Geaga JA, Drost DJ, Williamson PC, Rajakumar N, Sui Y, Dutton RA, Toga AW, Thompson PM. Mapping corpus callosum deficits in autism: an index of aberrant cortical connectivity. *Biological Psychiatry*. 2006; 60(3):218–25. [PubMed: 16460701]
111. Koldewyn K, Yendiki A, Weigelt S, Gweon H, Julian J, Richardson H, Malloy C, Saxe R, Fischl B, Kanwisher N. Differences in the right inferior longitudinal fasciculus but no general disruption of white matter tracts in children with autism spectrum disorder. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111(5):1981–6. [PubMed: 24449864]
112. Casanova, MF., Pickett, J. The Neuropathology of Autism. In: Casanova, MF., El-Baz, A., Suri, JS., editors. *Imaging the Brain in Autism*. Springer; New York: 2013. p. 27–43.
113. Leviton A, Gilles F. Ventriculomegaly, delayed myelination, white matter hypoplasia, and “periventricular” leukomalacia: how are they related? *Pediatric Neurology*. 1996; 15(2):127–36. [PubMed: 8888047]
114. Movsas TZ, Pinto-Martin JA, Whitaker AH, Feldman JF, Lorenz JM, Korzeniewski SJ, Levy SE, Paneth N. Autism Spectrum Disorder Is Associated with Ventricular Enlargement in a Low Birth Weight Population. *Journal of Pediatrics*. 2013
115. Piven J, Arndt S, Bailey J, Haverkamp S, Andreasen NC, Palmer P. An MRI study of brain size in autism. *Am J Psychiatry*. 1995; 152(8):1145–9. [PubMed: 7625461]
116. Page LA, Daly E, Schmitz N, Simmons A, Toal F, Deeley Q, Ambery F, McAlonan GM, Murphy KC, Murphy DG. In vivo 1H-magnetic resonance spectroscopy study of amygdala-hippocampal and parietal regions in autism. *American Journal of Psychiatry*. 2006; 163(12):2189–92. [PubMed: 17151175]
117. Naushad SM, Jain JM, Prasad CK, Naik U, Akella RR. Autistic children exhibit distinct plasma amino acid profile. *Indian Journal of Biochemistry and Biophysics*. 2013; 50(5):474–8. [PubMed: 24772971]
118. Kuwabara H, Yamasue H, Koike S, Inoue H, Kawakubo Y, Kuroda M, Takano Y, Iwashiro N, Natsubori T, Aoki Y, Kano Y, Kasai K. Altered metabolites in the plasma of autism spectrum disorder: a capillary electrophoresis time-of-flight mass spectroscopy study. *PLoS One*. 2013; 8(9):e73814. [PubMed: 24058493]
119. Horder J, Lavender T, Mendez MA, O’Gorman R, Daly E, Craig MC, Lythgoe DJ, Barker GJ, Murphy DG. Reduced subcortical glutamate/glutamine in adults with autism spectrum disorders: a [(1)H] MRS study. *Transl Psychiatry*. 2013; 3:e279. [PubMed: 23838890]
120. Bejjani A, O’Neill J, Kim JA, Frew AJ, Yee VW, Ly R, Kitchen C, Salamon N, McCracken JT, Toga AW, Alger JR, Levitt JG. Elevated glutamatergic compounds in pregenual anterior cingulate in pediatric autism spectrum disorder demonstrated by 1H MRS and 1H MRSI. *PLoS One*. 2012; 7(7):e38786. [PubMed: 22848344]

121. Shimmura C, Suda S, Tsuchiya KJ, Hashimoto K, Ohno K, Matsuzaki H, Iwata K, Matsumoto K, Wakuda T, Kamenoy Y, Suzuki K, Tsujii M, Nakamura K, Takei N, Mori N. Alteration of plasma glutamate and glutamine levels in children with high-functioning autism. *PLoS One*. 2011; 6(10):e25340. [PubMed: 21998651]
122. Choudhury PR, Lahiri S, Rajamma U. Glutamate mediated signaling in the pathophysiology of autism spectrum disorders. *Pharmacology, Biochemistry and Behavior*. 2012; 100(4):841–9.
123. Kondapalli KC, Hack A, Schushan M, Landau M, Ben-Tal N, Rao R. Functional evaluation of autism-associated mutations in NHE9. *Nat Commun*. 2013; 4:2510. [PubMed: 24065030]
124. Chih B, Engelman H, Scheiffele P. Control of excitatory and inhibitory synapse formation by neuroligins. *Science*. 2005; 307(5713):1324–8. [PubMed: 15681343]
125. Baron-Cohen S, Ring HA, Bullmore ET, Wheelwright S, Ashwin C, Williams SC. The amygdala theory of autism. *Neuroscience and Biobehavioral Reviews*. 2000; 24(3):355–64. [PubMed: 10781695]
126. Cahill L, Uncapher M, Kilpatrick L, Alkire MT, Turner J. Sex-related hemispheric lateralization of amygdala function in emotionally influenced memory: an fMRI investigation. *Learning and Memory*. 2004; 11(3):261–6. [PubMed: 15169855]
127. Ruigrok AN, Salimi-Khorshidi G, Lai MC, Baron-Cohen S, Lombardo MV, Tait RJ, Suckling J. A meta-analysis of sex differences in human brain structure. *Neuroscience and Biobehavioral Reviews*. 2014; 39:34–50. [PubMed: 24374381]
128. Schumann CM, Hamstra J, Goodlin-Jones BL, Lotspeich LJ, Kwon H, Buonocore MH, Lammers CR, Reiss AL, Amaral DG. The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. *Journal of Neuroscience*. 2004; 24(28):6392–401. [PubMed: 15254095]
129. Schumann CM, Barnes CC, Lord C, Courchesne E. Amygdala enlargement in toddlers with autism related to severity of social and communication impairments. *Biological Psychiatry*. 2009; 66(10):942–9. [PubMed: 19726029]
130. Troyb E, Rosenthal M, Eigsti IM, Kelley E, Tyson K, Orinstein A, Barton M, Fein D. Executive functioning in individuals with a history of ASDs who have achieved optimal outcomes. *Child Neuropsychol*. 2014; 20(4):378–97. [PubMed: 23731181]
131. Ravizza SM, Solomon M, Ivry RB, Carter CS. Restricted and repetitive behaviors in autism spectrum disorders: the relationship of attention and motor deficits. *Development and Psychopathology*. 2013; 25(3):773–84. [PubMed: 23880391]
132. Rosenthal M, Wallace GL, Lawson R, Wills MC, Dixon E, Yerys BE, Kenworthy L. Impairments in real-world executive function increase from childhood to adolescence in autism spectrum disorders. *Neuropsychology*. 2013; 27(1):13–8. [PubMed: 23356593]
133. Christ SE, Kester LE, Bodner KE, Miles JH. Evidence for selective inhibitory impairment in individuals with autism spectrum disorder. *Neuropsychology*. 2011; 25(6):690–701. [PubMed: 21728431]
134. Lemon JM, Gargaro B, Enticott PG, Rinehart NJ. Executive functioning in autism spectrum disorders: a gender comparison of response inhibition. *Journal of Autism and Developmental Disorders*. 2011; 41(3):352–6. [PubMed: 20506000]
135. Scott-Van Zeeland AA, Dapretto M, Ghahremani DG, Poldrack RA, Bookheimer SY. Reward processing in autism. *Autism Res*. 2010; 3(2):53–67. [PubMed: 20437601]
136. Rezaie P, Dean A. Periventricular leukomalacia, inflammation and white matter lesions within the developing nervous system. *Neuropathology*. 2002; 22(3):106–32. [PubMed: 12416551]
137. Kelly FJ, Fussell JC. Linking ambient particulate matter pollution effects with oxidative biology and immune responses. *Ann N Y Acad Sci*. 2015; 1340:84–94. [PubMed: 25716617]
138. Baburamani AA, Supramaniam VG, Hagberg H, Mallard C. Microglia toxicity in preterm brain injury. *Reproductive Toxicology*. 2014; 48:106–12. [PubMed: 24768662]
139. Harry GJ, Kraft AD. Microglia in the developing brain: a potential target with lifetime effects. *Neurotoxicology*. 2012; 33(2):191–206. [PubMed: 22322212]
140. Chew LJ, Fusar-Poli P, Schmitz T. Oligodendroglial alterations and the role of microglia in white matter injury: relevance to schizophrenia. *Developmental Neuroscience*. 2013; 35(2-3):p 102–29.

141. El Waly B, Macchi M, Cayre M, Durbec P. Oligodendrogenesis in the normal and pathological central nervous system. *Front Neurosci.* 2014; 8:145. [PubMed: 24971048]
142. Liu XB, Shen Y, Plane JM, Deng W. Vulnerability of premyelinating oligodendrocytes to white-matter damage in neonatal brain injury. *Neurosci Bull.* 2013; 29(2):229–38. [PubMed: 23456565]
143. Volpe JJ, Kinney HC, Jensen FE, Rosenberg PA. The developing oligodendrocyte: key cellular target in brain injury in the premature infant. *International Journal of Developmental Neuroscience.* 2011; 29(4):423–40. [PubMed: 21382469]
144. Schwarz JM, Sholar PW, Bilbo SD. Sex differences in microglial colonization of the developing rat brain. *Journal of Neurochemistry.* 2012; 120(6):948–63. [PubMed: 22182318]
145. Tabata H, Yoshinaga S, Nakajima K. Cytoarchitecture of mouse and human subventricular zone in developing cerebral neocortex. *Experimental Brain Research.* 2012; 216(2):161–8. [PubMed: 22080150]
146. Tramontin AD, Garcia-Verdugo JM, Lim DA, Alvarez-Buylla A. Postnatal development of radial glia and the ventricular zone (VZ): a continuum of the neural stem cell compartment. *Cerebral Cortex.* 2003; 13(6):580–7. [PubMed: 12764031]
147. Shigemoto-Mogami Y, Hoshikawa K, Goldman JE, Sekino Y, Sato K. Microglia enhance neurogenesis and oligodendrogenesis in the early postnatal subventricular zone. *Journal of Neuroscience.* 2014; 34(6):2231–43. [PubMed: 24501362]
148. Bird CR, Hedberg M, Drayer BP, Keller PJ, Flom RA, Hodak JA. MR assessment of myelination in infants and children: usefulness of marker sites. *AJNR Am J Neuroradiol.* 1989; 10(4):731–40. [PubMed: 2505502]
149. Vincze A, Mazlo M, Seress L, Komoly S, Abraham H. A correlative light and electron microscopic study of postnatal myelination in the murine corpus callosum. *Int J Dev Neurosci.* 2008; 26(6):575–84. [PubMed: 18556167]
150. Mengler L, Khmelinskii A, Diedenhofen M, Po C, Staring M, Lelieveldt BP, Hoehn M. Brain maturation of the adolescent rat cortex and striatum: changes in volume and myelination. *Neuroimage.* 2014; 84:35–44. [PubMed: 23994458]
151. Leviton A, Gressens P. Neuronal damage accompanies perinatal white-matter damage. *Trends Neurosci.* 2007; 30(9):473–8. [PubMed: 17765331]
152. Pandya DN., Seltzer, B. The topography of commissural fibers. In: Lepore, F.Pitito, M., Jasper, M., editors. *Two Hemispheres - One Brain: Functions of the Corpus Callosum.* Liss; New York: 1986. p. 47-73.
153. Barbas H, Pandya DN. Topography of commissural fibers of the prefrontal cortex in the rhesus monkey. *Experimental Brain Research.* 1984; 55(1):187–91. [PubMed: 6745350]
154. Paul LK, Brown WS, Adolphs R, Tysza JM, Richards LJ, Mukherjee P, Sherr EH. Agenesis of the corpus callosum: genetic, developmental and functional aspects of connectivity. *Nat Rev Neurosci.* 2007; 8(4):287–99. [PubMed: 17375041]
155. Narberhaus A, Segarra D, Caldu X, Gimenez M, Pueyo R, Botet F, Junque C. Corpus callosum and prefrontal functions in adolescents with history of very preterm birth. *Neuropsychologia.* 2008; 46(1):111–6. [PubMed: 17897687]
156. Ewing-Cobbs L, Prasad MR, Swank P, Kramer L, Mendez D, Treble A, Payne C, Bachevalier J. Social communication in young children with traumatic brain injury: relations with corpus callosum morphometry. *Int J Dev Neurosci.* 2012; 30(3):247–54. [PubMed: 21807088]
157. Shah PS, Balkhair T. Air pollution and birth outcomes: a systematic review. *Environ Int.* 2011; 37(2):498–516. [PubMed: 21112090]
158. Stieb DM, Chen L, Eshoul M, Judek S. Ambient air pollution, birth weight and preterm birth: a systematic review and meta-analysis. *Environmental Research.* 2012; 117:100–11. [PubMed: 22726801]
159. Lener MS, Wong E, Tang CY, Byne W, Goldstein KE, Blair NJ, Haznedar MM, New AS, Chemerinski E, Chu KW, Rimsky LS, Siever LJ, Koenigsberg HW, Hazlett EA. White matter abnormalities in schizophrenia and schizotypal personality disorder. *Schizophr Bull.* 2015; 41(1): 300–10. [PubMed: 24962608]

160. Langevin LM, Macmaster FP, Crawford S, Lebel C, Dewey D. Common white matter microstructure alterations in pediatric motor and attention disorders. *Journal of Pediatrics*. 2014; 164(5):1157–1164 e1. [PubMed: 24576693]
161. Chaim TM, Zhang T, Zanetti MV, da Silva MA, Louza MR, Doshi J, Serpa MH, Duran FL, Caetano SC, Davatzikos C, Busatto GF. Multimodal magnetic resonance imaging study of treatment-naïve adults with attention-deficit/hyperactivity disorder. *PLoS One*. 2014; 9(10):e110199. [PubMed: 25310815]
162. Newbury AJ, Rosen GD. Genetic, morphometric, and behavioral factors linked to the midsagittal area of the corpus callosum. *Front Genet*. 2012; 3:91. [PubMed: 22666227]
163. Paul LK. Developmental malformation of the corpus callosum: a review of typical callosal development and examples of developmental disorders with callosal involvement. *J Neurodev Disord*. 2011; 3(1):3–27. [PubMed: 21484594]
164. Gilliam M, Stockman M, Malek M, Sharp W, Greenstein D, Lalonde F, Clasen L, Giedd J, Rapoport J, Shaw P. Developmental trajectories of the corpus callosum in attention-deficit/hyperactivity disorder. *Biological Psychiatry*. 2011; 69(9):839–46. [PubMed: 21247556]
165. Qiu MG, Ye Z, Li QY, Liu GJ, Xie B, Wang J. Changes of brain structure and function in ADHD children. *Brain Topography*. 2011; 24(3-4):243–52. [PubMed: 21191807]
166. Schnoebelen S, Semrud-Clikeman M, Pliszka SR. Corpus callosum anatomy in chronically treated and stimulant naïve ADHD. *J Atten Disord*. 2010; 14(3):256–66. [PubMed: 20460495]
167. Cao Q, Sun L, Gong G, Lv Y, Cao X, Shuai L, Zhu C, Zang Y, Wang Y. The macrostructural and microstructural abnormalities of corpus callosum in children with attention deficit/hyperactivity disorder: a combined morphometric and diffusion tensor MRI study. *Brain Research*. 2010; 1310:172–80. [PubMed: 19852946]
168. Cheung C, Yu K, Fung G, Leung M, Wong C, Li Q, Sham P, Chua S, McAlonan G. Autistic disorders and schizophrenia: related or remote? An anatomical likelihood estimation. *PLoS One*. 2010; 5(8):e12233. [PubMed: 20805880]
169. King BH, Lord C. Is schizophrenia on the autism spectrum? *Brain Research*. 2011; 1380:34–41. [PubMed: 21078305]
170. Meyer U, Feldon J, Dammann O. Schizophrenia and autism: both shared and disorder-specific pathogenesis via perinatal inflammation? *Pediatric Research*. 2011; 69(5 Pt 2):26R–33R.
171. Rapoport J, Chavez A, Greenstein D, Addington A, Gogtay N. Autism spectrum disorders and childhood-onset schizophrenia: clinical and biological contributions to a relation revisited. *Journal of the American Academy of Child and Adolescent Psychiatry*. 2009; 48(1):10–8. [PubMed: 19218893]
172. Barneveld PS, Pieterse J, de Sonnevile L, van Rijn S, Lahuis B, van Engeland H, Swaab H. Overlap of autistic and schizotypal traits in adolescents with Autism Spectrum Disorders. *Schizophrenia Research*. 2011; 126(1-3):231–6. [PubMed: 20933368]
173. Brown MS, Singel D, Hepburn S, Rojas DC. Increased glutamate concentration in the auditory cortex of persons with autism and first-degree relatives: a (1)H-MRS study. *Autism Res*. 2013; 6(1):1–10. [PubMed: 23166003]
174. Shimmura C, Suzuki K, Iwata Y, Tsuchiya KJ, Ohno K, Matsuzaki H, Iwata K, Kamenoy Y, Takahashi T, Wakuda T, Nakamura K, Hashimoto K, Mori N. Enzymes in the glutamate-glutamine cycle in the anterior cingulate cortex in postmortem brain of subjects with autism. *Mol Autism*. 2013; 4(1):6. [PubMed: 23531457]
175. Sodhi MS, Sanders-Bush E. Serotonin and brain development. *International Review of Neurobiology*. 2004; 59:111–74. [PubMed: 15006487]
176. de Lacy N, King BH. Revisiting the relationship between autism and schizophrenia: toward an integrated neurobiology. *Annu Rev Clin Psychol*. 2013; 9:555–87. [PubMed: 23537488]
177. Smoller JW, Craddock N, Kendler K, Lee PH, Neale BM, Nurnberger JI, Ripke S, Santangelo S, Sullivan PF. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet*. 2013; 381(9875):1371–9. [PubMed: 23453885]
178. Palmen SJ, Hulshoff Pol HE, Kemner C, Schnack HG, Durston S, Lahuis BE, Kahn RS, Van Engeland H. Increased gray-matter volume in medication-naïve high-functioning children with autism spectrum disorder. *Psychological Medicine*. 2005; 35(4):561–70. [PubMed: 15856726]

179. Sanfilippo M, Lafargue T, Arena L, Rusinek H, Kushner K, Lautin A, Loneragan C, Vaid G, Rotrosen J, Wolkin A. Fine volumetric analysis of the cerebral ventricular system in schizophrenia: further evidence for multifocal mild to moderate enlargement. *Schizophrenia Bulletin*. 2000; 26(1):201–16. [PubMed: 10755682]
180. Shen MD, Nordahl CW, Young GS, Wootton-Gorges SL, Lee A, Liston SE, Harrington KR, Ozonoff S, Amaral DG. Early brain enlargement and elevated extra-axial fluid in infants who develop autism spectrum disorder. *Brain*. 2013; 136(Pt 9):2825–35. [PubMed: 23838695]
181. Elitt CM, Rosenberg PA. The challenge of understanding cerebral white matter injury in the premature infant. *Neuroscience*. 2014; 276:216–38. [PubMed: 24838063]
182. Volpe JJ. Cerebral white matter injury of the premature infant-more common than you think. *Pediatrics*. 2003; 112(1 Pt 1):176–80. [PubMed: 12837883]
183. Back SA. Perinatal white matter injury: the changing spectrum of pathology and emerging insights into pathogenetic mechanisms. *Ment Retard Dev Disabil Res Rev*. 2006; 12(2):129–40. [PubMed: 16807910]
184. Melhem ER, Hoon AH Jr, Ferrucci JT Jr, Quinn CB, Reinhardt EM, Demetrides SW, Freeman BM, Johnston MV. Periventricular leukomalacia: relationship between lateral ventricular volume on brain MR images and severity of cognitive and motor impairment. *Radiology*. 2000; 214(1): 199–204. [PubMed: 10644124]
185. Perlman JM. White matter injury in the preterm infant: an important determination of abnormal neurodevelopment outcome. *Early Human Development*. 1998; 53(2):99–120. [PubMed: 10195704]
186. Wang S, Fan G, Xu K, Wang C. Potential of diffusion tensor MR imaging in the assessment of cognitive impairments in children with periventricular leukomalacia born preterm. *European Journal of Radiology*. 2013; 82(1):158–64. [PubMed: 23084875]
187. Back SA, Rosenberg PA. Pathophysiology of glia in perinatal white matter injury. *Glia*. 2014; 62(11):1790–815. [PubMed: 24687630]
188. Guo S, Kendrick KM, Zhang J, Broome M, Yu R, Liu Z, Feng J. Brain-wide functional inter-hemispheric disconnection is a potential biomarker for schizophrenia and distinguishes it from depression. *Neuroimage Clin*. 2013; 2:818–26. [PubMed: 24179833]
189. Gilmore JH, van Tol JJ, Lewis Streicher H, Williamson K, Cohen SB, Greenwood RS, Charles HC, Kliewer MA, Whitt JK, Silva SG, Hertzberg BS, Chescheir NC. Outcome in children with fetal mild ventriculomegaly: a case series. *Schizophrenia Research*. 2001; 48(2-3):219–26. [PubMed: 11295375]
190. Gilmore JH, van Tol J, Kliewer MA, Silva SG, Cohen SB, Hertzberg BS, Chescheir NC. Mild ventriculomegaly detected in utero with ultrasound: clinical associations and implications for schizophrenia. *Schizophrenia Research*. 1998; 33(3):133–40. [PubMed: 9789905]
191. Fannon D, Tennakoon L, Sumich A, O'Ceallaigh S, Doku V, Chitnis X, Lowe J, Soni W, Sharma T. Third ventricle enlargement and developmental delay in first-episode psychosis: preliminary findings. *British Journal of Psychiatry*. 2000; 177:354–9. [PubMed: 11116778]
192. Gilmore JH, Smith LC, Wolfe HM, Hertzberg BS, Smith JK, Chescheir NC, Evans DD, Kang C, Hamer RM, Lin W, Gerig G. Prenatal mild ventriculomegaly predicts abnormal development of the neonatal brain. *Biological Psychiatry*. 2008; 64(12):1069–76. [PubMed: 18835482]
193. Kyriakopoulou V, Vatansever D, Elkommos S, Dawson S, McGuinness A, Allsop J, Molnar Z, Hajnal J, Rutherford M. Cortical Overgrowth in Fetuses With Isolated Ventriculomegaly. *Cerebral Cortex*. 2013
194. Bockhorst KH, Narayana PA, Liu R, Ahobila-Vijjula P, Ramu J, Kamel M, Wosik J, Bockhorst T, Hahn K, Hasan KM, Perez-Polo JR. Early postnatal development of rat brain: in vivo diffusion tensor imaging. *J Neurosci Res*. 2008; 86(7):1520–8. [PubMed: 18189320]
195. Sturrock RR. Myelination of the mouse corpus callosum. *Neuropathology and Applied Neurobiology*. 1980; 6(6):415–20. [PubMed: 7453945]
196. Garcia GJ, Schroeter JD, Kimbell JS. Olfactory deposition of inhaled nanoparticles in humans. *Inhal Toxicol*. 2015; 27(8):394–403. [PubMed: 26194036]
197. Pedata P, Stoeger T, Zimmermann R, Peters A, Oberdorster G, D'Anna A. Are we forgetting the smallest, sub 10 nm combustion generated particles? *Particle and Fibre Toxicology*. in press.

198. Ronkko T, Virtanen A, Kannosto J, Keskinen J, Lappi M, Pirjola L. Nucleation mode particles with a nonvolatile core in the exhaust of a heavy duty diesel vehicle. *Environmental Science and Technology*. 2007; 41:6384–6389. [PubMed: 17948783]

Highlights

- Male UFP-treated mice showed persistent ventriculomegaly and microglial activation
- Male UFP-treated mice showed reduced CC size and hypomyelination
- UFP exposures also produced excitatory-inhibitory imbalance only in male mice

Figure 1.

CAPS exposure leads to male specific and time dependent increases in IBA-1. IBA-1 levels (group mean $\pm$ S.E.) as percent of time point control plotted separately for males and females for Air and CAPS-treated groups at PND14 and PND55 in anterior commissure and hippocampus (**Panel A**) and at PND 270 in corpus callosum (**Panel B**). CAPS = concentrated ambient ultrafine particles; PND=postnatal day; Air-14= brains from Air exposed offspring at postnatal day 14; Air-55=brains from Air exposed offspring at postnatal day 55; CAPS-14= brains collected at postnatal day 55 from offspring exposed to concentrated ambient ultrafine particles; CAPS-55= brains collected at postnatal day 14 from offspring exposed to concentrated ambient ultrafine particles; brains collected at postnatal day 55 from offspring exposed to concentrated ambient ultrafine particles; * = significantly different from corresponding time point control. N=4-5/group.

Figure 2.

CAPS exposure leads to increase in lateral ventricle size; effects were graded with the example demonstrating the most severe effects shown here. **Panels A-C:** Rostral to caudal section of **Air** exposed males at PND 14 with lateral ventricle (LV) becoming visible indicated by arrowhead in Panel C. **Panels D-E** Matched Rostral to caudal sections of CAPS-treated males at PND14. Note: LV and CC damage apparent rostrally; Arrowheads in Panel D shows bridge across ventricle (fusion) and coarctation in LV in Panel E. Magnification 2×; CAPS = concentrated ambient ultrafine particles; PND=postnatal day; FCtx=frontal cortex; Cpu=caudate putamen. n=4-5/group.

Figure 3.

CAPS exposure leads to male specific increased ventricular size. Lateral ventricle area (group mean $\pm$ S.E) of males (left panel) and females (right panel) treated with Air or postnatal CAPS were measured at three time points: PND 14 (defined 24 hr post exposure), PND 55 and PND 270; $n=4-6$ /group, average of at least 3 sections/brain. CAPS = concentrated ambient ultrafine particles; PND=postnatal day. Male data modified from [80] and female data unpublished.

Figure 4.

CAPS treatment reduces size of CC in both sexes at PND14 with a trend towards greater magnitude effects in males. **Panels A–D:** CC size (μm^2) of individual male and female mice following Air or postnatal CAPS treatment as measured at PND14 and PND270. Each data point represents an individual value. Sample sizes of 3–5 for PND14 and 7–11 for PND270. **Panel E.** Group mean $\pm$ S.E. size of CC across time points for male and females in treatment groups as indicated. CAPS = concentrated ambient ultrafine particles; PND=postnatal day; CC=corpus callosum.

Figure 5.

CAPS treatment induces male specific CC hypomyelination at PND14. **Panels A-D.**

Fluorescent intensity of myelin basic protein labeling expressed as percent of total CC area at PND14 (Panels A,B) and PND270 (Panels C,D) for male and female Air vs. postnatal CAPS-treated mice as measured at 3 different sites along the CC: truncus, medial external capsule and lateral external capsule for treatment groups as indicated. Shown is group mean $\pm$ S.E. **Panels E-G.** Relative area of MBP labeling across time points for male and females in treatment groups as indicated for truncus, medial external capsule (MEC) and lateral external capsule (LEC), respectively. Treatment $\times$ sex $\times$ time point indicates significant 3-way interaction in overall ANOVAs. Sample sizes of 3-5 for PND14 and 4-11 for PND270. Shown is group mean $\pm$ S.E. CAPS = concentrated ambient ultrafine particles; PND=postnatal day; CC=corpus callosum.

Figure 6.

CAPS exposure does not alter striatal myelination in either sex. **Panels A-B:** Fluorescent intensity of myelin basic protein labeling expressed as arbitrary units in striatum at PND14 and PND270, respectively, for male and female Air vs. postnatal CAPS-treated mice. **Panel C:** Trajectory of density of myelin basic protein staining between PND14 and PND270 for males and females for indicated treatment groups. Sample sizes of 3-5 for PND14 and 4-11 for PND270. Shown is group mean $\pm$ S.E. CAPS = concentrated ambient ultrafine particles; PND=postnatal day.

Figure 7.

CAPS treatment does not alter frontal cortex myelination in either sex. **Panels A-B:** Density of myelin basic protein staining (μm^2) in frontal cortex at PND14 and PND270, respectively, for male and female Air vs. postnatal CAPS-treated mice. **Panel C:** Trajectory of density of myelin basic protein staining between PND14 and PND270 for males and females for indicated treatment groups. Sample sizes of 3-5 for PND14 and 4-11 for PND270. Data are group mean $\pm$ S.E. CAPS = concentrated ambient ultrafine particles; PND=postnatal day.

Figure 8.

CAPS produced a male specific frontal cortex excitatory/inhibitory imbalance. Glutamate/GABA ratios based on levels of frontal cortex at PND14 and PND60 as based on extracellular glutamate, glutamine and GABA in Air vs. postnatal CAPS treated males (left panel) and female (right panel) mice. CAPS = concentrated ambient ultrafine particles; PND=postnatal day; Treatment= main effect of CAPS in statistical analysis; timepoint=main effect of time point in statistical analysis, n=9-12/group. Data modified from [80] and [81]. Shown are group means $\pm$ S.E

Figure 9.

CAPS exposure increased GFAP labeling in amygdala. Fluorescent intensity of GFAP labeling in amygdala in male mice exposed to postnatal CAPS at PND14 and normalized to Air exposed controls. $n=5/\text{gp}$. Shown is group mean $\pm$ S.E. CAPS = concentrated ambient ultrafine particles; PND=postnatal day; GFAP=glial fibrillary acidic protein.

Figure 10.

Cumulative records of individual male from indicated treatment groups on the fixed ratio (FR) wait schedule. Y axis response counter resets to baseline after each 30 responses; pips (backslashes) = reward delivery, horizontal lines = periods of waiting, vertical lines = FR responding. Modified from [79].