EFFECT OF PRENATAL ORGANIC SOLVENT EXPOSURE ON STRUCTURAL CONNECTIVITY AT CHILDHOOD

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ABSTRACT

Glycol ethers are part of organic solvents. They are used in industry and at home during manufacturing or usage of products such as paints, cleaning agents and cosmetics. The specific detection of subtle, low-dose effects of early-life exposure to these solvents on neuropsychological performance in children is a trendy subject of investigation. Neuroimaging allows looking into brain function and identifying different cerebral connections that may be affected by these neurotoxicants. In this paper, we investigated the specific effects of prenatal low-level exposure to different glycol ethers, on brain development of children between 10 and 12 years old. Based on previous studies suggesting cognitive disabilities in the attention, inhibition and working memory, we proposed a structural connectivity analysis using graph theory restricted to the regions involved in these functions. Our results suggest a possible relationship between the attention, working memory and inhibition and prenatal exposure to specific glycol ethers, such as ethoxyacetic acid, ethoxyethoxyacetic acid and 2-butoxyacetic acid.

Index Terms— *diffusion-weighted imaging, graph theory, diffusion, organic solvents, brain development.*

1. INTRODUCTION

Glycol ethers are part of organic solvents. They are used in industry and the home during manufacturing or usage of products such as paints, cleaning agents and cosmetics. Pregnant women are ubiquitously exposed to organic solvents and more particularly glycol ethers according to French and European biomonitoring data [1]. The neurotoxicity of glycol ethers is recognized in adults [2]. And these substances are able to cross the blood brain barrier [3], which may interfere directly in neurological brain development [4], raising particular concern. Specific effects of prenatal exposure to glycol ether on child neurodevelopment have been poorly studied. Only one epidemiological study investigated the specific effects of prenatal exposure to glycol ethers on neurodevelopment. Authors report that 6-year old children with the higher level of in-utero exposure to glycol ethers had lower verbal comprehension and copying score [1]. However, converging evidence shows an association between prenatal exposures to organic solvents in general and child neurodevelopment in occupational setting [5-8]. Some previous studies reported cognitive and behavioral deficits, including hyperactivity, attention deficits, lower processing speed and memory scores, reduced dexterity and visual-motor coordination) [1, 6, 9-12]. Moreover, the organic solvents may induce complex cerebral changes, resulting in the neurobehavioral dysfunction that are poorly understood. Advanced neuroimaging can provide increased sensitivity to measure such deficits and identify cerebral connections that are affected by these neurotoxicants.

Diffusion-weighted magnetic resonance imaging is a noninvasive measuring the extent of water diffusion. Diffusion-weighted imaging (DWI) can quantitatively assess the microstructural characteristics of white matter (WM) tracts [13, 14]. Specifically, diffusion MRI tractography methods have been developed to map out major WM pathways, allowing mapping of the brain's structural connectivity in vivo [15]. Graph theoretical analysis provides a powerful way of quantifying neural pathways and the structure of the connectomes [16]. This approach consists in modeling the brain as a complex network where nodes are associated with regions of interest and an edge strength represents the degree of connectivity between a pair of regions. Network-based algorithms provide parameters that define the global organization of the brain and its alterations within the network. The disruption of brain connectivity was shown to play a central role in the pathophysiology of several neurological and psychiatric illnesses, such as the cortico–striato–thalamo–cortical network, reward circuits and limbic system [17].

In this paper, we investigated the specific effects of prenatal exposure to some low-level glycol ethers, called alkoxycarboxylic acids, on brain development in children aged between 10 and 12 years old. Based on previous studies suggesting cognitive disabilities in attention, inhibition and working memory networks, we proposed a structural connectivity analysis using graph theory in regions involved in these functions. Identifying the neural correlates underlying the exposure of each glycol ethers will help identify the microstructural modifications in exposed children.

2. MATERIAL

2.1. Population

Participants were recruited from the PELAGIE prospective cohort study of mothers and their newborn infants who were born between 2002 and 2006 [18]. This cohort included 3421 women at the beginning of pregnancy recruited in general population. The children were followed during their development. Environmental as well as health condition data were collected. For the MRI project, a sub-cohort was randomly selected and children had to be born at term (delivery after 35 weeks of amenorrhea) and to present no major condition at birth (neonatal hospitalization, hypoglycemia, 5 minute-Apgar score <7), no maternal tobacco or alcohol consumption during pregnancy, no exposure to medical treatment during childhood which could affect their neurodevelopment (methylphenidate, psychotropic or antiepileptic drugs, etc.). Finally, 100 children and parents agreed to participate in a new study 10 years later. The appropriate ethics committees approved the study (Committee for the protection of persons- n°2013-A01420-45, French Consulting Committee for the Treatment of Information in Medical Research, n°09.485, and the French National Commission for the Confidentiality of Computerized Data, n°909347), and written informed consents were obtained from all subjects and parents.

2.2. Data

All children underwent MR imaging using a 3T MR Scanner (Magnetom Verio, VB17, Siemens, Erlangen, Germany) using a 32-channel receiver head coil. The 3D T1-weighted images were acquired covering the whole brain (160 sagittal slices) with TR = 1.9s, TE = 2.26 ms, flip angle= 9°, in-plane resolution = 256 × 256, FOV = 256 mm ×256 mm and thickness =1 mm). The DWI data were gathered on 60 slices using an interleaved slice acquisition order, slice thickness of 2 mm, no gap, in-plane resolution = 2 mm × 2 mm and in a 256 mm × 256 mm field of view, TR/TE = 11 000/99 ms, flip angle was 90°, pixel bandwidth =1698 Hz/px, using 30 directions and a b-value of 1000 s/mm^2.

At inclusion, 73 women had returned a urine sample (first morning void) in a 10 mL test tube (95 × 16 mm polypropylene, with wing plug). Six alkoxycarboxylic acids (glycol ether metabolites) of the most used glycol ethers in France at the time of inclusion were analyzed: methoxyacetic acid (MAA), ethoxyacetic acid (EAA), ethoxyethoxyacetic acid (EEAA), 2-butoxyacetic acid (BAA), phenoxyacetic acid (PhAA) and 2-methoxypropionic acid (2-MPA). Procedure details of chemical analyzes are described elsewhere [1]. The children had prenatal exposures to some low-level glycol ethers [1] and nevertheless, were categorized into 3 groups based on tertiles: low, moderate or high-level groups.

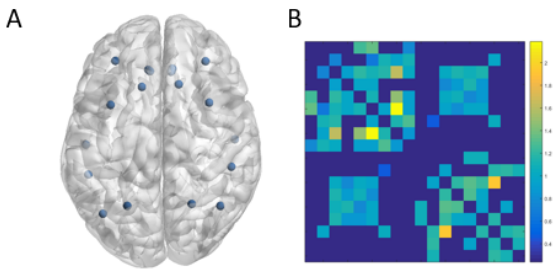

Figure 1: (A) Graphical representation of the nodes and (B) the mean connectivity matrix of the participants.

2.3. Image processing

Preprocessing of diffusion images was mainly performed using the open source medical image processing toolbox Anima (https://github.com/Inria-Visages/Anima-Public/wiki). Diffusion images were corrected for eddy current-induced image distortion using Block-Matching Distortion Correction method ensuring an opposite symmetric transformation [19]. Then, rigid realignment was then performed to compensate for subject motion and ensure voxel-to-voxel correspondence across gradients. Then, denoising step using blockwise non-local means filtering was applied [20]. Skull stripping was performed using an atlas-registration-based method. Whole brain WM tractography was performed using fiber assignment by continuous tracking (FACT) method [15]. Then, the individual T1-w images were coregistered to the b = 0 images in the DWI space [21]. The T1 weighted images from the included subjects were preprocessed using FreeSurfer [22] for cortical and subcortical gray matter parcellation. Our analysis was restricted to regions including in the motor inhibition, attention and working memory networks (as displayed in Figure 1.A.), such as dorsolateral prefrontal cortex and intraparietal sulcus, anterior cingulate, supplementary motor area, inferior and frontal gyrus, frontal eye fields, ventral frontal cortex and temporoparietal junction. These regions were estimated using the Freesurfer segmentation.

3. METHODS

3.1. Network construction

To construct connectivity matrices, we used the restricted segmentation to define the graph nodes (18 regions). For each subject group, we constructed a corresponding 18 × 18 brain connectivity matrix with each element (i,j) depicting the number of fibers that passes through regions i and j (as displayed in Figure 1.B.), normalized by the mean volume of the two regions. . The group-averaged matrices were thresholded to include only connections found in at least 75% of subjects, in order to suppress false positive fibers arising from tractography errors [23].

3.2. Network analysis

To localize specific pairs of brain regions in which structural connectivity was modified by organic solvents, a two-sample Student t-test was performed after controlling for age and gender between low- and high-level groups for every solvent. A primary threshold ($p < 0.01$) was first applied to the t-statistic to define a set of connections. To estimate the significance for each of them, a nonparametric permutation approach (10,000 permutations) was applied. Data was randomly reallocated into two groups (high- or low-level groups) and the t-statistic was computed independently for each connection. The corrected p-value was determined by finding the fraction of t-statistics that were at least as high as the original (non-permuted) statistic.

4. RESULTS

We observed statistically significant difference in connectivity between left ventral frontal cortex and superior parietal cortex (t-statistic= -2.6 and p-value=0.01) between children with high (n=23) and low (n=26) exposure to BAA. We reported decreased fiber density in regions involved in attention network for children whose mothers had highest levels of BAA. This suggested that there was a possible relationship between the attention network and BAA. Statistically significant difference was found in fiber density between the caudal middle frontal cortex and the frontal cortex (rostal midlle and superior parts) (t-value= -2.3/-2.5 and p-value=0.01/0.02) between groups of low (n=28) and high (n=22) level of EEAA exposure. These regions are involved in working memory. An association between some connections of inhibition and attention network and EEAA exposure was found. The 20 children whose mothers had high levels of EEAA exhibited higher fiber density between left anterior cingulate cortex and inferior frontal region, as well as ventral frontal and superior temporal cortices in comparison with 30 low-level children. No statistical association was found between structural connectivity and level exposure of PhAA and MAA. Concerning the MPA, we observed decreased fiber density in the high-level group compared with the low level one in different connections involved in attention such as bilateral ventral frontal cortex and temporo-parietal junction. However, the statistics did not survive permutation test.

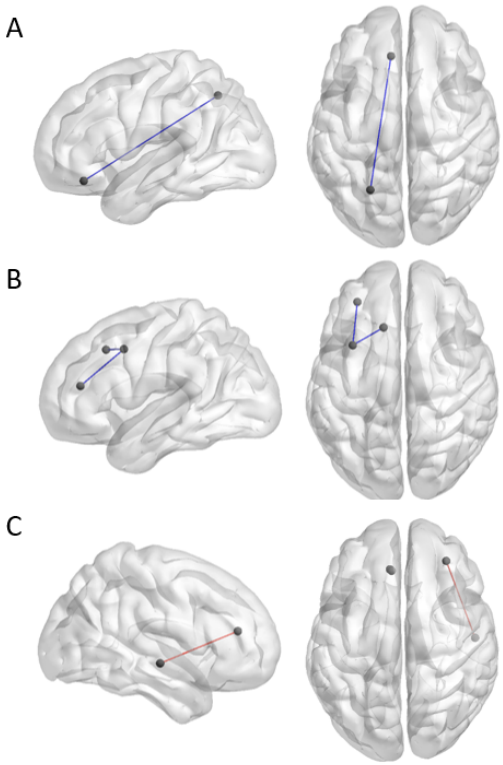

Figure 2: Regions exhibited significant between-group differences in fiber density for BAA (A), EAA (B) and EEAA (C). The blue color represented the higher values for children whose mothers had high level of exposure to glycol ethers ($p<0.05$ with permutation test).

5. DISCUSSION

To our knowledge, this is the first structural connectivity study analyzing the specific effects of prenatal low-level exposure to different glycol ethers. This preliminary study suggests that prenatal organic solvent exposure, at levels observed with routine use and below the threshold for any signs of acute exposure, has a measureable effect on brain connectivity in a sample of children between 10 and 12 years old. Our findings exhibit a decreased fiber density in prefrontal and parietal cortices associated with higher prenatal glycol ether exposure group, suggesting defects in the brain circuitry underlying performance of attention, working memory and motor inhibition. These results are appeared to be in line with studies reported association between prenatal exposures to organic solvents in general and neurocognitive deficits [1, 6, 9-12]. We also observed the same affected regions than the ones found for adults

chronically highly exposed to solvents [24]. Moreover, in this study, children whose mothers had high levels of EEAA exhibited higher fiber density between two pairs of regions. This elevated structural connectivity could provide a compensatory mechanism favoring cognitive performance.

6. CONCLUSION

In this paper, for the first time, we were able to investigate the specific effects of prenatal low-level exposure to different glycol ethers on brain development using a structural connectivity analysis. Our results suggest that there was a possible relationship between the attention, working memory and inhibition networks and specific glycol ethers, such as BAA, EAA and EEAA. Given that this study presents limits in term of exposure measure and other confounder factors might be explored, these results need to be confirmed by other studies on potential neurotoxicity of glycol ethers. Nevertheless, our observations provide insights for future studies and suggest the interest of this kind of investigation.

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