Parietal Dysfunction in Children with Prenatal Alcohol Exposure

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Abstract

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The parietal lobe has been shown to be one of the regions most affected by prenatal alcohol exposure. Functional domains dependent on intact parietal functioning, including mathematical and visuospatial ability, have been consistently implicated in fetal alcohol spectrum disorders. This thesis examines, in children, using blood oxygenation level dependent (BOLD) functional Magnetic Resonance Imaging, the effect of prenatal alcohol exposure on brain activation during symbolic and nonsymbolic number processing, and place learning in a virtual environment. These functional domains were investigated using tasks of proximity judgment and exact addition to assess neural correlates of symbolic number processing in 65 children (mean age ± SD = 9.45 ± 0.42 years), nonsymbolic number comparison at varying difficulties in 34 children (11.55 ± 1.15 years), and place learning in a virtual reality computer generated (CG) arena in 57 children (9.44 ± 0.42 years; 29 boys).

In the symbolic number processing tasks greater prenatal alcohol exposure was related to less activation in the right horizontal intraparietal sulcus known to mediate mental representation and manipulation of quantity. Children with fetal alcohol syndrome and partial fetal alcohol syndrome appeared to compensate for this deficit by increased activation of the left angular gyrus during the proximity judgment task.

Syndromal children with fetal alcohol syndrome or partial fetal alcohol syndrome also demonstrated poor recruitment of the right horizontal intraparietal sulcus during nonsymbolic number comparison, indicating that mental representation and manipulation of quantity are impaired in children with heavy prenatal alcohol exposure, irrespective of the representation format used. This impairment was compensated for by the left angular gyrus, with only exposed children needing to recruit the left angular gyrus to a greater extent as number comparison task difficulty increased. Further, reduced activation of the right posterior superior parietal lobule in children with increasing prenatal alcohol exposure suggests that exposed children may be less able to employ the attentional systems associated with number processing. Notably, activation of nonsyndromal heavily exposed children was impaired in the right posterior superior parietal lobule, but spared in the right horizontal intraparietal sulcus.

In boys *only*, prenatal alcohol exposure was associated with poorer place learning and reduced activation during place learning in the precuneus and posterior cingulate, as well as parahippocampal gyrus, frontal and temporal lobes, caudate, insula, claustrum, lentiform nucleus and thalamus. In girls, prenatal alcohol exposure was not associated with place learning performance or activation during place learning in any regions. These results confirm that boys and girls use different navigation strategies that rely on different brain regions and suggest that the regions used by boys are more susceptible to alcohol damage, while the regions used by girls are relatively spared.

In conclusion, all the tasks investigated showed prenatal alcohol exposure related alterations in parietal function, with the impairments being widespread throughout the parietal lobe bilaterally. Notably, activation of the bilateral precuneus was affected by prenatal alcohol exposure in both the spatial navigation and nonsymbolic number comparison tasks. It is possible that this is a key region linking the deficits in number processing and visuospatial skills in children with prenatal alcohol exposure.

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To my brother, Drew, thanks for always believing in me.

To Mom and Dad, who are always supportive and encouraging, many thanks for everything you do for me and for being there for me no matter what.

List of Abbreviations

Abbreviation	Description
AA	absolute alcohol
ADHD	attention-deficit/hyperactivity disorder
AG	angular gyrus
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ARND	alcohol related neurodevelopmental disorder
BA	Brodmann area
BOLD	blood oxygenation level dependent
CG Arena	Computer-Generated Arena
CUBIC	Cape Universities Brain Imaging Centre
DD	developmental dyscalculia
EA	exact addition
EA_CTL	control block in the exact addition task
FAS	fetal alcohol syndrome
FASD	fetal alcohol spectrum disorders
fMRI	functional magnetic resonance imaging
GLM	general linear model
HE	heavily exposed
HRF	haemodynamic response function
IPS	intraparietal sulcus
JSAIS	Junior South African Individual Scales
LSD	least-squares difference
MPRAGE	magnetization prepared rapid gradient echo
MRI	magnetic resonance imaging
MWM	Morris water maze
NC	nonsymbolic number comparison
PAE	prenatal alcohol exposure
PET	positron emission tomography
PFAS	partial fetal alcohol syndrome
PJ	proximity judgment
PJ_CTL	control block in the proximity judgment task

PSPL	posterior superior parietal lobule
ROI	region of interest
SD	standard deviation
SES	socioeconomic status
SNARC	spatial-numerical association of response codes
TBM	tensor-based morphometry
TOJ	temporal order judgment
TS	Turner syndrome
UCT	University of Cape Town
VBM	voxel-based morphometry
WISC-III	Wechsler Intelligence Scale for Children, Third Edition
WISC-IV	Wechsler Intelligence Scale for Children, Fourth Edition
WRAML	Wide Range Assessment of Memory and Learning

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Preface

This thesis uses neuroimaging to examine parietal dysfunction in children with prenatal alcohol exposure as it pertains specifically to number processing and spatial navigation. It includes three independent manuscripts that investigate, using functional magnetic resonance imaging (fMRI), the effect of prenatal alcohol exposure on the neural correlates of, respectively, symbolic number processing, nonsymbolic number processing, and place learning in a computer generated virtual environment. These articles are found in chapters 3 to 5. These functional domains were selected due to their dependence on intact parietal functioning. The first two papers examined the effect of prenatal alcohol exposure on the activation of predefined regions of interest in the parietal lobe known to be critically involved in number processing. As brain regions associated with spatial navigation are less well understood, the third article used a whole brain analysis to examine effects of prenatal alcohol exposure on the neural correlates of spatial location memory during a virtual reality spatial navigation task. The combination of three independent articles into the thesis does lead to necessary repetition as each article in itself consists of an introduction, methods, results and discussion.

Chapter 1 presents a motivation for the study. Fetal alcohol spectrum disorders (FASD) is introduced and neuroimaging studies investigating FASD are briefly reviewed, with a specific emphasis on studies of number processing and place learning in virtual environments.

Chapter 2 contains background information relating to fMRI and the effects of prenatal alcohol exposure on the parietal lobe, number processing and visuospatial processing.

The first article, which has been published in the journal Neuroimage: Clinical, is in chapter 3. In this article, fMRI was used to investigate the effect of prenatal alcohol exposure on the activation of predefined regions of interest within the parietal lobe during two different symbolic number processing tasks, proximity judgment and exact addition. This article was co-authored by Ernesta M. Meintjes, Christopher D. Molteno, Sandra W. Jacobson and Joseph L. Jacobson.

Ernesta Meintjes provided overall project supervision, contributed to the design of the study, and reviewed the paper. Sandra and Joseph Jacobson designed the study, recruited subjects, performed interviews and neurobehavioural assessment, provided suggestions regarding data analysis and interpretation, and reviewed the paper. Christopher Molteno administered maternal interviews, which included socio-demographic information and alcohol, smoking and drug use.

The second article, which forms chapter 4, used fMRI during a nonsymbolic number comparison task of varying difficulty levels to examine prenatal alcohol exposure effects on the same regions of interest. This manuscript is currently being reviewed by the co-authors, Joseph L. Jacobson, Christopher D. Molteno, Sandra W. Jacobson and Ernesta M. Meintjes, and has not yet been submitted for publication.

The third article is included as chapter 5. This paper involves a whole brain fMRI analysis that examines the effects of prenatal exposure on the neural correlates of place learning in a virtual environment in girls and boys separately. This article is under review by the other authors: Sandra W. Jacobson, Kevin G.F. Thomas, Christopher D. Molteno, Joseph L. Jacobson and Ernesta M. Meintjes.

Chapter 6 contains a discussion of the study as a whole. The role of the parietal lobe in number processing and visuospatial skills is discussed, as well as behavioural links between number and spatial processing. Our results are explored in more detail, comparing effects of prenatal alcohol exposure on brain activation during number processing and place learning, and between symbolic and nonsymbolic number processing, to highlight similarities and differences and possible reasons for these. Finally, the limitations of this study and possible future work are described and conclusions are drawn.

Chapter 1 : Introduction

Prenatal alcohol exposure causes impairment in brain structure and function, leading to cognitive and behavioral deficits. Impairments in general intelligence [1, 2], attention [3], learning and memory [3-5], executive functioning [3, 6, 7], language [3], motor skills [3, 8], cognitive processing speed [1, 9-11], visuospatial processing [3, 5], and number processing [1, 2, 12-16] have been reported.

Fetal alcohol syndrome (FAS), the most severe of the fetal alcohol spectrum disorders (FASD), is characterized by distinctive craniofacial dysmorphology (short palpebral fissures, thin upper lip (vermillion), flat philtrum), small head circumference and pre- and/or postnatal growth retardation [17]. In partial FAS (PFAS), some of the craniofacial dysmorphology is seen, as well as either small head circumference, retarded growth, or neurobehavioral deficits. Heavily exposed (HE) individuals lacking the distinctive dysmorphology are diagnosed with alcohol related neurodevelopmental disorder (ARND) if they exhibit cognitive and/or behavioral impairment [18]. The reported incidence of FASD in the Western Cape Province of South Africa is amongst the highest in the world [19]. In some Western Cape communities, the prevalence of FASD is 20-28%, in comparison to a prevalence of 2-5% in the USA and Europe [19].

The effect of prenatal alcohol exposure was first documented in the medical literature in 1968 [20] and the term FAS was introduced in 1973 [21], but until the recent introduction of neuroimaging techniques, most of the knowledge about the associated brain abnormalities came from autopsy studies. Since 1992, neuroimaging techniques have been used to advance our understanding of the more subtle structural and functional alterations related to prenatal alcohol exposure [22].

Numerous studies have examined structural alterations associated with FASD. These have included studies of whole brain volume (eg. [23-31]); regional volumes (eg. [23, 25-30, 32-36]), including both tensor-based morphometric (TBM) [37] and voxel-based morphometric (VBM) [38] methods; cortical thickness (eg. [24, 39-42]); shape abnormalities (eg. [36, 43-45]); and cortical gyrification [46]. Structurally, the parietal lobe has been shown to be one of the most alcohol affected regions in the brain (eg. [29, 30, 38, 42, 45]).

The parietal lobe plays a key role in many domains, including visuospatial processing [47], number processing [48-50], language processing [51], response inhibition [52, 53], non-spatial working memory [54], episodic memory [55], alertness [56], task switching [57], attention [58] and time perception [59].

As such, it is not surprising that functional domains dependent on intact parietal functioning, including mathematical [14, 16, 60] and visuospatial ability [61], have been consistently implicated in FASD.

In comparison to structural MRI studies, there have been relatively few functional MRI (fMRI) studies of FASD. The author could only find 22 papers using fMRI to investigate effects of prenatal alcohol exposure on brain function prior to

the work included in this thesis. Four of the papers investigated intrinsic connectivity [62-65] and the remaining 18 task based activation, including studies of response inhibition [66-68], attention [69, 70], visual working memory [71], verbal working memory [72, 73], verbal learning [74], finger tapping [75], spatial working memory [76-80], and number processing [60, 81]. Alterations in parietal activation have been reported in response inhibition [66-68], attention [70], memory [71, 72, 74, 78, 79], and number processing [60, 81].

Despite the large number of behavioural studies that have examined effects of FASD on number processing (eg. [12, 16, 60, 81-84]), the only two using fMRI [60, 81] both addressed symbolic number processing (eg. Arabic numbers or sequences of words). Neural correlates of nonsymbolic number processing (eg. numbers of dots) have not been investigated in FASD. Further, neither of these previous studies using fMRI was able to assess dose dependent effects, nor the effect of task difficulty.

Performance deficits in place learning in virtual environments have also been reported in FASD [61, 85], but the neural correlates have only been investigated in a small pilot study performed by our group [86], which compared only the most severely affected children with FAS/PFAS to control children and did not investigate associations with degree of alcohol exposure, or differences in nonsyndromal alcohol exposed children.

The aim of this study was to perform a comprehensive investigation of parietal dysfunction in children with prenatal alcohol exposure by examining differences in brain activation using blood oxygenation level dependent (BOLD) fMRI during symbolic and nonsymbolic number processing, and place learning in a virtual environment. These functional domains were investigated using tasks of proximity judgment and exact addition to assess neural correlates of symbolic number processing, nonsymbolic number comparison at varying difficulties, and place learning in a virtual reality Computer-Generated (CG) arena.

Chapter 2 : Background

2.1. Fetal Alcohol Spectrum Disorder

2.1.1. The Effect of Prenatal Alcohol Exposure on the Parietal Lobe

The parietal lobe is one of the four major lobes of the brain. It is covered by the parietal bone and is situated posterior to the frontal lobe, anterior to the occipital lobe and superior to the temporal lobe (see Figure 2.5). The postcentral sulcus divides the parietal lobe into the postcentral gyrus and the posterior parietal cortex. The lateral posterior parietal cortex is divided into the inferior parietal lobule and superior parietal lobule by the intraparietal sulcus. The inferior parietal lobule contains the supramarginal gyrus and angular gyrus. The medial part of the posterior parietal cortex comprises the precuneus and the posterior cingulate/retrosplenial cortex.

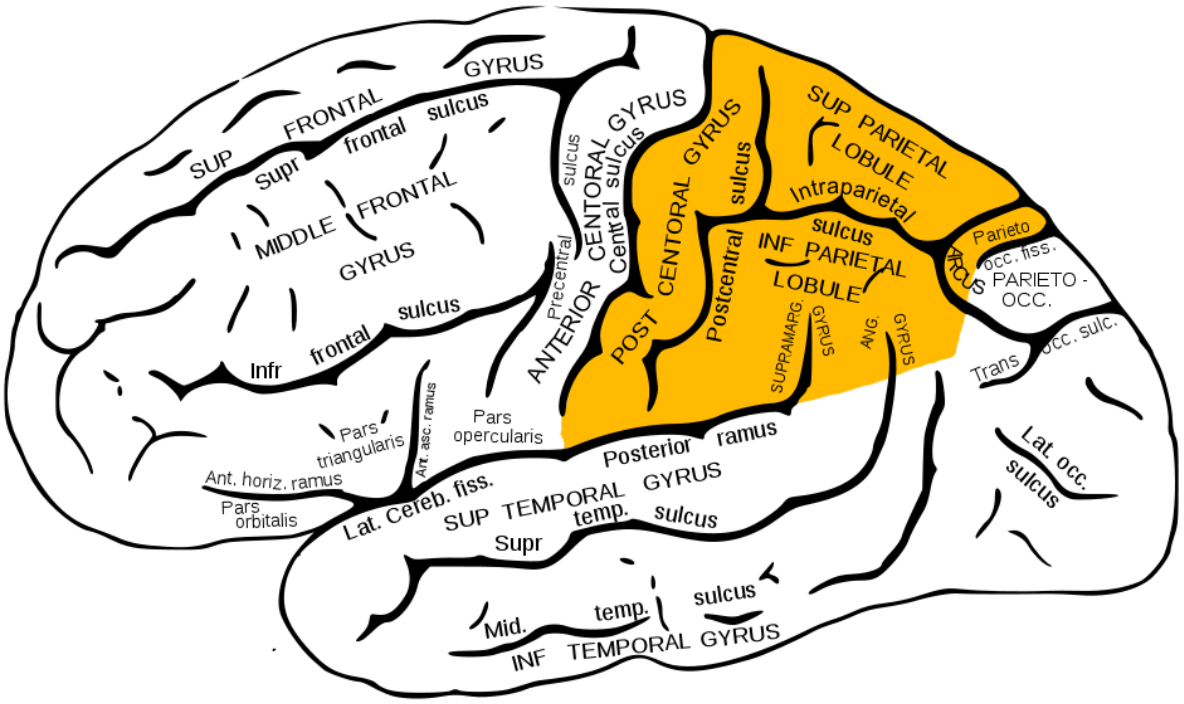

Figure 2.1: The parietal lobe. (adapted from [87])

The parietal lobe has been shown previously to be particularly vulnerable to effects of prenatal alcohol exposure, with volume reductions persisting even after controlling for alcohol-related reductions in whole brain volume. Both parietal grey and white matter volumes are disproportionately reduced [30, 45].

Prenatal alcohol exposure has been associated with reductions in the precuneus and superior parietal lobe [37]. The bilateral inferior parietal regions have been found to be narrower and have greater grey matter density in participants with prenatal alcohol exposure compared to controls [8]. A similar left posterior temporo-parietal region was found to have too much grey matter and too little white matter in the same FASD subjects [7]. Studies of the effect of prenatal alcohol exposure on cortical thickness have been inconsistent. One study found only thickness decreases associated with FASD, including the bilateral superior parietal lobule [40], while others found only FASD related thickness increases, including the lateral parietal cortices and the left inferior parietal lobule [39, 41, 42]. One of the reasons for the discrepancy in these findings could be the different ages of the participants [24]. Group differences are not necessarily stationary, but may vary over time, highlighting the need for longitudinal studies.

A longitudinal study of cortical volume showed that the developmental trajectories of inferior and superior parietal regions differed between controls and alcohol exposed subjects. Control subjects showed more curvature to their development, with volume increases and then decreases over time, while exposed subjects showed less change in volume [29].

White Matter Microstructure Alterations

Diffusion Tensor Imaging (DTI) uses the diffusion characteristics of water molecules in brain tissue to evaluate the integrity of white matter. Fractional anisotropy (FA), the extent to which water preferentially moves parallel to the axonal axis, has been found to be reduced in both the left and right parietal lobes in children with prenatal alcohol exposure [88, 89], suggesting compromised parietal white matter integrity. In addition, higher FA in the left parietal white matter, directly underlying the IPS, has been shown to be related to better math performance in children with prenatal alcohol exposure [90]. In the same regions, greater parallel diffusivity, which is associated with axonal integrity, was also associated with higher math scores.

Functional Alterations

The activation of a wide range of parietal regions has been implicated in individuals with prenatal alcohol exposure, with exposed subjects showing both activation increases and decreases in comparison to control subjects.

Response inhibition tasks shown mainly increased activation in exposed groups, with increases being seen in the inferior parietal lobule, supramarginal gyrus, postcentral gyrus and the precuneus [66-68]. These activation increases have been attributed to greater effort during inhibition [66] or an immature pattern of inhibitory processing [68]. In contrast, the postcentral gyrus shows activation decreases in exposed individuals during cued trials, possibly as a result of failure to make use of the predictive pattern [68].

Tasks involving attention demonstrate less of an increase in activation of the superior parietal lobule, posterior cingulate and postcentral gyrus with increasing task difficulty in exposed individuals [70]. This reduction has been attributed to

the exposed group being incapable of recruiting additional neural resources to cope with the more difficult condition [70].

Activation increases have been found in exposed groups in the inferior parietal lobule and superior parietal lobule during verbal memory tasks [72, 74]. The superior and inferior parietal lobules also show increased activation during spatial location memory, as does the precuneus [79]. These activation increases have been attributed to inefficient processing [72] and greater effort [79] requiring additional neural resources by exposed individuals. In contrast, during an *n*-back visual working memory task the posterior parietal lobe is activated less by exposed groups during the 2-back condition [71]. This weaker activation was not evident during the easier 1-back condition, and while the exposed group performed worse than the control group during both the 1-back and 2-back conditions, the difference was more pronounced in the more difficult 2-back condition. The difference in activation was attributed to a "ceiling effect", where exposed individuals were unable to recruit additional neural resources to cope with the more difficult 2-back condition [71].

During mathematical tasks, exposed groups have demonstrated both activation increases and decreases in parietal regions compared to controls. Activation decreases in the inferior parietal lobule, superior parietal lobule and posterior horizontal intraparietal sulcus were attributed to poorer functioning of the number processing network [60, 81]. The increases in activation seen in the precuneus, posterior cingulate, angular gyrus and anterior horizontal intraparietal sulcus were attributed to increased effort or the fact that exposed individuals employ verbal strategies to compensate for deficits in the number processing network [60].

2.1.2. FASD and Number Processing

Mathematical ability seems to be especially sensitive to the effect of prenatal alcohol exposure, and mathematical deficits remain, even after controlling for effects of alcohol on IQ [1, 2, 12-15]. Although this impairment is well established, little work has been done to investigate which components of number processing are impaired.

Kopera-Frye *et al.* [83] investigated number processing in adolescents and adults with FASD using a test battery aimed at determining the components of number processing that are impaired. The tasks investigated included number reading and writing, approximate and exact arithmetic, proximity judgment (Which of 2 numbers is closer to a third number e.g. "Is 3 or 6 closer to 5?"), number comparison and cognitive estimation (where the subject makes an estimate of the numerical measure of an object e.g. "What is the normal length of a bus?"). Though individuals with FASD were unimpaired in number reading and writing, they were impaired in more complex tasks, such as calculation and cognitive estimation. However, the pattern of impairments varied significantly between individuals, with the most subjects impaired in cognitive estimation and approximate subtraction, and only very few impaired in number comparison and proximity judgment, both of which involve symbolic magnitude comparison.

In contrast, Jacobson *et al.* [91] found that in children prenatal alcohol exposure was more strongly related to number comparison and proximity judgment than to exact or approximate arithmetic. Although approximate subtraction was impaired in children with prenatal alcohol exposure, its relation to prenatal alcohol exposure was completely mediated by its relation to magnitude comparison (number comparison and proximity judgment). It was concluded that mathematical difficulties in children with FASD is related to a specific deficit in magnitude representation. In their later study of adolescents [16], they obtained similar results, again demonstrating that difficulties in mathematical abilities in adolescents with prenatal alcohol exposure is attributable to impairment of the core number system.

2.1.3. FASD and Visuospatial Deficits

Prenatal alcohol exposure has been shown to impair spatial location memory [4, 77, 79, 92-98] and place learning during virtual navigation [61, 85].

There is evidence that prenatal alcohol exposure affects spatial location memory in "table top tests". Uecker *et al*. [92, 93] found that children with FAS are more impaired in memory for object location than in the memory for the objects themselves. Kaemingk *et al*. [94] similarly found a specific impairment in spatial location memory. Prenatal alcohol exposure related impairment in spatial location memory has also been demonstrated in the Visual Learning task from the Wide Range Assessment of Memory and Learning (WRAML) [4, 95, 99] and spatial *n*-back tasks [77-79].

Compared to spatial location memory, the effect of prenatal alcohol exposure on spatial navigation has received little attention. In contrast, it has been widely explored in rodents [100-102]. Many of these studies used the Morris water maze (MWM) [103], which is the gold standard for testing place learning ability in non-primate mammals [104]. In a MWM, the animals are required to learn the position of a platform submerged in water, beginning in different positions around the tank. It has been found that prenatal alcohol exposure impairs rodents' place learning abilities in the MWM [100-102].

More recently, the effects of prenatal alcohol exposure on place learning in humans has been investigated using computer simulated 3D virtual versions of the Morris water maze (VWM) [105]. Children prenatally exposed to alcohol performed worse during a probe trial [85] and travelled a greater distance to reach a hidden platform [61].

To the author's knowledge, only one pilot study has examined the effects of prenatal alcohol exposure on brain activation during spatial navigation [86]. This study was performed in a small subsample of children using a desktop based virtual environment, known as the Computer Generated (CG) Arena [106-108]. Although not significant, FAS/PFAS children travelled a greater distance to reach a hidden platform. Despite the absence of significant differences in performance, children with FAS/PFAS activated frontal, left inferior parietal and cerebellar regions more than control children during passive place learning suggesting that greater effort was required by the alcohol exposed children to perform at a level similar to the control children.

2.1.4. Cape Town FASD cohorts

In this study, data were acquired from children in two separate cohorts, namely the fMRI cross-sectional cohort and the Cape Town longitudinal cohort. In the former, children were recruited retrospectively, while mothers were recruited prospectively during pregnancy for the latter. Children in both cohorts are from the Cape Coloured (mixed ancestry) community in Cape Town, South Africa, where the incidence of FASD is exceptionally high due to poor socioeconomic circumstances and historical practices of compensating farm labour with wine, which have contributed to a tradition of heavy recreational weekend binge drinking [19].

This study included 65 right-handed 8- to 12-year-old children from the fMRI cross-sectional cohort, of whom 40 had been heavily exposed to alcohol prenatally and 25 were non-exposed controls in the same age range. Thirty-seven children were the older siblings of participants in our Cape Town longitudinal cohort [73]. The others were identified by screening all of the 8- to 12-year-old children from an elementary school in a rural section of Cape Town, where there is a very high incidence of alcohol abuse among local farm workers [60].

Here, 86 right-handed 8- to 12- year old children from the Cape Town longitudinal cohort were included. Of these, 59 had been heavily exposed to alcohol prenatally and 27 were non-exposed controls in the same age range. Their mothers, from the same community as the cross-sectional cohort, were recruited between 1999 and 2002 at their first antenatal clinic visit [73].

Each mother was interviewed regarding her alcohol consumption during pregnancy, to determine incidence and amount of drinking on a day-by-day basis during a typical week during pregnancy. Any child whose mother reported consuming at least 14 standard drinks per week (1.0 oz AA⁄day) on average or engaged in binge drinking during pregnancy (4 or more drinks⁄occasion) was considered heavily exposed. Controls were children whose mothers reported abstaining or drinking less than 7 drinks per week with no binge drinking during pregnancy.

Each child was examined for growth and FAS dysmorphology by two expert U.S.-based dysmorphologists during a clinic conducted in 2005; a subset of children who could not attend the clinic was examined by a Cape Town-based dysmorphologist [73]. There was substantial agreement among the examiners on the assessment of all dysmorphic features, including the three principal fetal alcohol-related features—philtrum and vermilion measured on the Astley and Clarren [109] rating scales and palpebral fissure length (median $r = 0.78$). Nine children met the revised Institute of Medicine criteria [17] for full FAS: at least two of the principal dysmorphic features, small head circumference (bottom 10^{th} percentile), and low weight or short stature (bottom 10th percentile). Nine met criteria for PFAS; that is, two features and at least one of the following: small head circumference, low weight, short stature, or low IQ (<70).

2.2. Functional magnetic resonance imaging (fMRI)

fMRI is an imaging technique that uses Magnetic Resonance Imaging (MRI) technology to indirectly measure neural activity by detecting signal changes associated with blood oxygenation. The fact that fMRI is noninvasive, does not involve exposure to radiation and is safe [110], makes it ideal for use in research. fMRI provides high spatial resolution (of the order of 1-3 mm in each direction), is able to image throughout the brain [110]. A disadvantage of fMRI is that it does not measure neural activity directly, but instead measures changes in signal that arise from changes in blood oxygenation from which neural activity is inferred [110]. Also, activation reflects mass neural activity, and not the activity of specific neuronal units [111]. In contrast to its high spatial resolution, fMRI has relatively low temporal resolution [110], of the order of seconds, as the slow haemodynamic response restricts its ability to capture rapid changes [112].

Blood oxygenation level dependent (BOLD) fMRI is the most commonly used form of fMRI and was discovered by Seiji Ogawa [113]. BOLD fMRI detects increases in relative blood oxygenation that accompanies neuronal activity. Neural activation requires energy, so when neurons fire, blood flow increases to supply the firing neurons with more oxygen and glucose to meet the increased metabolic demands. More oxygen is supplied than the neurons can use, resulting in an increase in the ratio of oxyhaemoglobin (oxygenated blood) to deoxyhaemoglobin (deoxygenated blood) in the region of neural activation. The change in the BOLD signal accompanying neural activation is a result of the different magnetic properties of oxyhaemoglobin and deoxyhaemoglobin. The magnetic susceptibility of oxyhaemoglobin is not very different from brain tissue and therefore causes little or no magnetic distortion. In contrast, deoxyhaemoglobin is paramagnetic and distorts the magnetic field causing signal loss. Thus the relative increase in oxyhaemoglobin relative to deoxyhaemoglobin results in less signal loss and an increase in the MR signal (Figure 2.1) [114, 115].

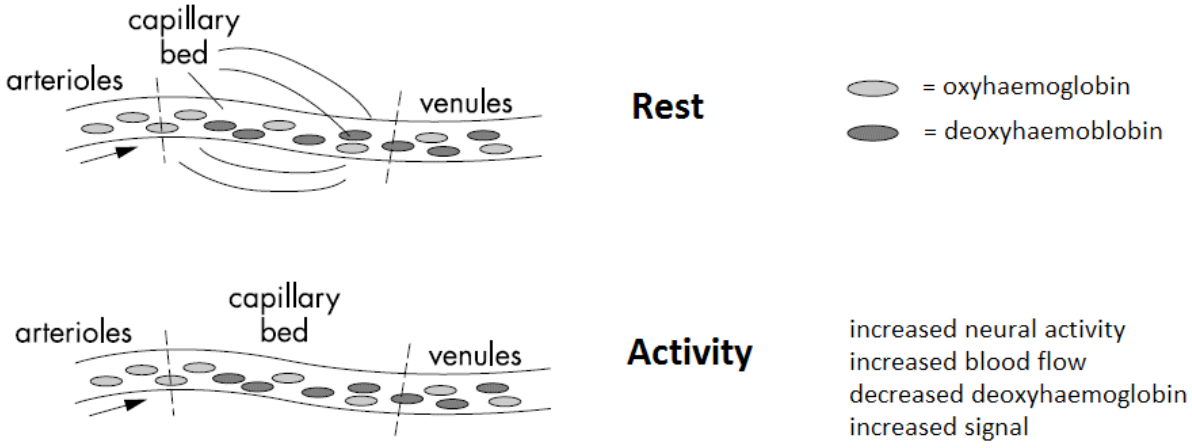

Figure 2.2: Schematic illustration of changes in haemodynamic variables resulting from neural activity. (adapted from [114])

In reality neuronal activation does not result in a simple increase in signal and there are various models of the haemodynamic response function (HRF), which is the assumed response of the MR signal to neuronal activation. A commonly used model is shown in Figure 2.2. The initiation of neural activation results in an initial dip in the MRI signal resulting from the decrease of oxyhaemoglobin before the inflow of blood. This dip is followed by an increase in signal due to the increased level of oxyhaemoglobin following increased blood flow. After the termination of the stimulus, the signal drops below baseline (post-stimulus undershoot), before returning to baseline [116]. This undershoot occurs because blood flow decreases more quickly than the blood volume, resulting in a greater level of deoxyhaemoglobin [117].

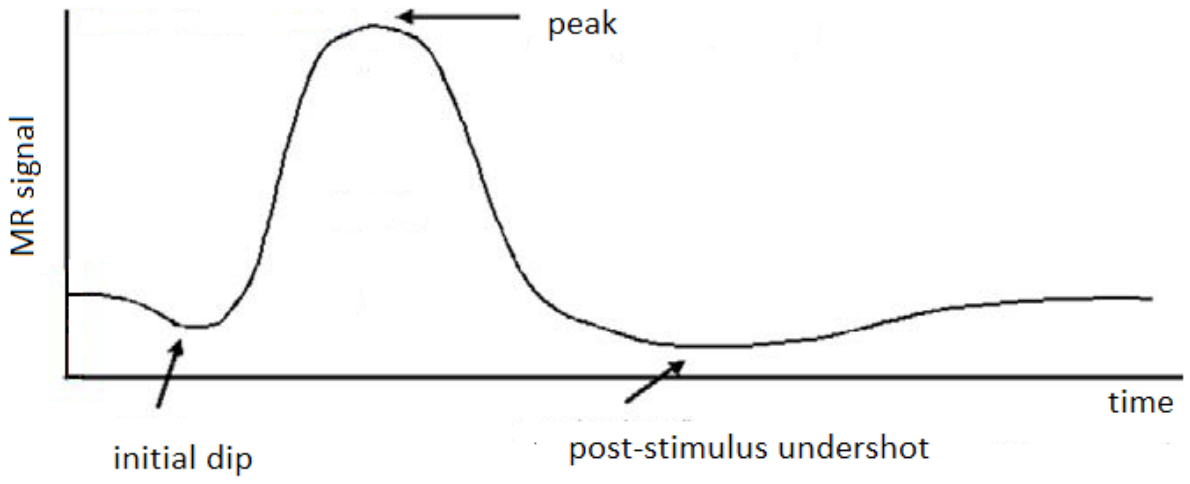

Figure 2.3: Haemodynamic response function (adapted from [116])

2.2.1. Task Based fMRI

In tasked based fMRI, a low resolution functional volume of the brain is acquired every few seconds while the subject is performing a series of tasks (conditions) interspersed with rest. The aim of task based fMRI is to identify the regions of the brain in which signal changes are temporally correlated with the task being performed.

The two major design types in task based fMRI are blocked design and event-related design. In blocked design, the design type initially used for fMRI, trials of the same type are grouped together into blocks of roughly 20 to 40 seconds. It is assumed that the signal reaches a steady state while performing similar tasks. Each individual trial is therefore not modelled separately, but the block as a whole. Blocked design gives high statistical power, so it is useful to use when looking at a task that is not expected to give a strong signal change. On the negative side, blocked design is sensitive to signal drift, for example caused by head motion, especially if there are just a few blocks [110]. All the tasks used in this study employed the blocked design.

In event-related design, each trial is modelled as a discrete event. The main advantage of event-related design is the ability to detect transient BOLD responses. It allows randomisation of conditions and variation in the time between stimulus presentations, reducing the subject's ability to predict when and what will happen and maintaining their attention level throughout the experiment. Using event-related design, incorrect trials can be modelled separately from correct trials. The downside of this method is that it has lower statistical power, so longer scans are needed [110].

When analysing fMRI data, changes in activation, rather than the actual level of activation is important. Because of this, blocks of rest are usually included in the experiment.

While a person is performing a task, their brain is still involved in processes not related to the process of interest, such as, for example, visual activity or motor responses. Because of this, control tasks are also often included to isolate the functional domain of interest. A control task is usually designed to differ from the actual task only in the process of interest. If a response is required during the task, it is good for the control task also to include a response, and the control task should try and match the task visually as much as possible. When comparing the activation between the task and the control task, the activation should be related to the functional domain of interest.

An alternative strategy is to include tasks differing in some other aspect, such as, for example, difficulty levels.

2.2.2. Preprocessing

In this project, all fMRI analyses were performed using BrainVoyager QX 2.6 (Brain Innovation, Maastricht, the Netherlands). BrainVoyager is coded in C++ and is highly optimized, making it very fast. Although easy to use, BrainVoyager is flexible enough to enable us to perform the statistical analyses we required [118].

Before statistical analysis, fMRI data usually undergoes a series of preprocessing steps. This preprocessing is necessary to prepare the data for statistical analysis, by making sure that certain assumptions about the data are met and aims to increase the signal to noise ratio to maximize the sensitivity of the statistical analysis.

The statistical analysis of the fMRI data assumes that the whole volume of the brain is measured at the same time while in reality it is acquired as a series of 2D slices over the repetition time of the sequence (typically about 2 s). Slice scan timing correction interpolates each voxel's time course to determine the signal at the beginning of the measurement [117]. Here we used cubic spline interpolation.

If a subject moves in the scanner, the signal time course within a voxel will be contaminated with signals from neighbouring voxels. Motion correction involves coregistering each volume to the first volume of the functional run to ensure that for every voxel the entire time course of the signal originates from the same anatomical region. Linear interpolation was used to estimate the 6 motion parameters (3 for translation and 3 for rotation), and sinc interpolation for motion correction. Although slow, sinc interpolation is recommended for motion correction because no spatial

smoothing is introduced, resulting in corrected functional data closely reflecting the original data [118]. The above process does not, however, fully remove motion artefacts due to spin history effects and the fact that motion during readout causes incorrect spatial encoding within a volume. For this reason data with severe motion should be excluded from all analyses. For adults, data with motion exceeding 1 mm translation in any direction or 1 degree rotation around any axis are typically discarded. In this study of children, the largest continuous section of data with no movement greater than 3 mm displacement or 3.0° rotation was analysed.

Spatial smoothing involves averaging the signal intensity of each voxel with its neighbouring voxels, and is performed on each functional volume separately. The aim of this blurring is to increase the signal to noise ratio by reducing noise but keeping the underlying signal of interest [110]. We used no spatial smoothing on our 3 T data, but spatially smoothed the 1.5 T data using a Gaussian filter (FWHM 4mm).

Temporal filtering is performed on each voxel's time course individually. A voxel's time course shows how the voxel's signal intensity changes over time. While some of this variance is due to changes in the task stimulus, the rest of the variance is due to factors unrelated to the stimulus, including scanner-related drifts, physiological effects, and high frequency noise. The aim of temporal filtering is to remove the unwanted components without removing the signal of interest [110]. High-pass filtering removes the low frequency noise, such as scanner drift and physiological effects, while low-pass filtering removes the high frequency noise. We used only a high pass filter of 2 cycles/point.

After the preprocessing of the functional data, the low resolution functional data needs to be aligned to the corresponding subject's high resolution anatomical MR scan. To combine results from multiple subjects, anatomical images (along with the co-registered functional data) need to be transformed into a common space, such as Talairach space which was used in this study [119].

2.2.3. fMRI Statistical Analysis

The general purpose of statistical analysis is to determine which regions show significant signal changes in response to the task stimulus. A model of the expected response is created, which is fit to the time course of the signal for every voxel in the brain using a general linear model (GLM).

To model the expected response, a stimulation protocol is defined that specifies the timing of the different task conditions during the fMRI acquisition. For each condition, except rest, a "box-car" time course is created, where the time points belonging to the condition are assigned the value of "1", while the rest of the time points are given the value "0" [118], see Figure 2.3. This box-car time course is convolved with the HRF (see Figure 2.4), and added to the model as a predictor. Usually the subject's motion profiles throughout the fMRI acquisition are added to the model as predictors of no interest as motion events may explain some of the variance in the data. In this work we Z-transformed each of the 6 motion parameter estimate time courses before adding them to the model.

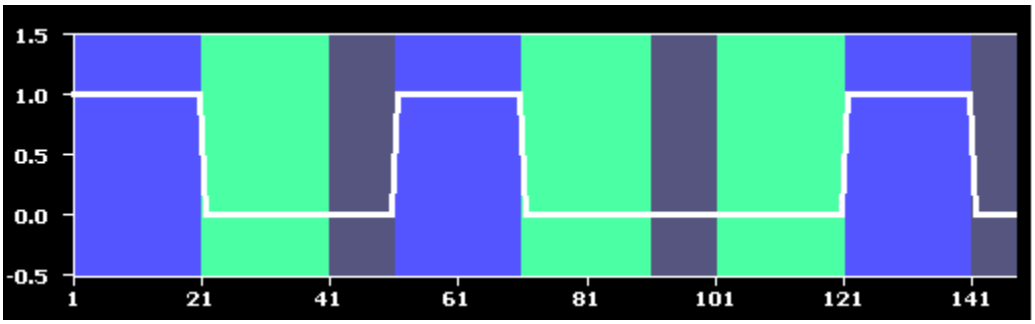

Figure 2.4: An example of a box-car time course for a single task condition.

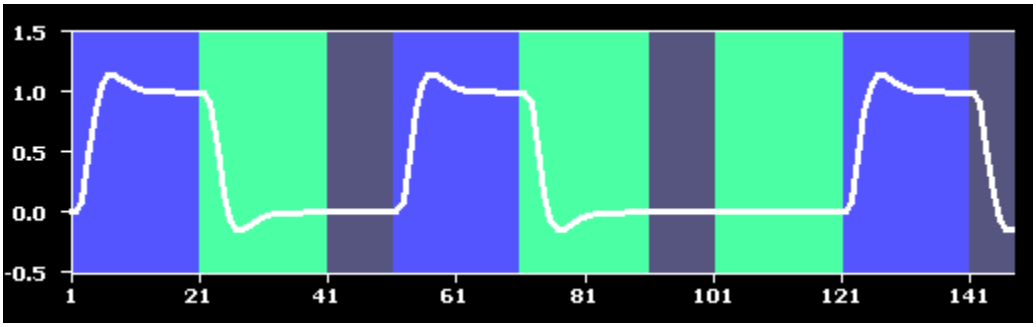

Figure 2.5: An example of a box-car time course convolved with the haemodynamic response function (HRF).

The GLM calculates, for every voxel, beta values for each predictor. The beta value specifies how much of the variance in the signal is explained by a particular predictor. If the absolute value of beta is high, much of the signal variance is associated with that task condition, while a low beta indicates that the brain activity is not explained by that condition [110].

Group analysis

A group analysis using random effects allows for the generalisation of the results to a population, and the comparison of activation between groups of subjects. A random effects analysis is performed on a voxel-by-voxel basis and is often performed in 2 separate stages. In the first stage, GLM parameters are estimated for each subject independently. The resulting first-level parameter estimates (betas) from each subject are then used as dependent variables in the 2nd analysis stage. The second level analysis uses a standard analysis of variance (ANOVA) approach, which can include finding group differences between parameter estimates from the first level analysis. In addition, other variables can be included as covariates, extending the ANOVA approach to an analysis of covariance (ANCOVA) [118]. For example, we performed a whole brain group analysis using random effects for our CG Arena place learning task. To find regions where activation was correlated with prenatal alcohol exposure, we included alcohol exposure as a covariate in the second level analysis.

The 2nd level analysis yields *p*-values based on the uncertainty of the 2nd level parameter estimates [110]. A common approach is to consider voxels with significance levels greater than a chosen threshold to be "activated". Since the statistical analysis is run separately on thousands of voxels, a multiple comparison problem arises. Using a *p*-value of 0.05, even if there was no task stimulus, by chance 5 000 out of the approximately 100 000 voxels in the brain would be falsely identified as being "active".

There are various methods for dealing with the multiple comparison problem. Bonferroni correction is a method commonly used to maintain strict control of the global error probability. In order to ensure a global error probability of *p*, a single voxel needs to be significant at a level of *p* divided by the number of voxels. This method tends to be overly conservative for neuroimaging data since it assumes that each test is independent, where in reality a voxel is not independent from neighbouring voxels [120]. In contrast, spatial extent methods are based on the fact that voxels are not activated individually, but rather clusters of voxels are activated together, which makes them more appropriate for neuroimaging data [121]. We used a cluster size correction based on Monte Carlo simulations. After applying an initial voxel-level threshold, this method uses Monte Carlo simulations to calculate the minimum cluster size considered significant at a global error probability for the selected *p*-value [122].

Region of interest analysis

An alternative to running a whole brain analysis is to perform a region of interest (ROI) analysis. In an ROI analysis, a collection of voxels is chosen and the GLM is run either on the signal time courses of every voxel within the ROI or on the average of the signal time courses of all voxels within the ROI. Due to the fact that the analyses are performed in far fewer voxels, the multiple comparison problem is severely reduced. The selection of ROIs is normally hypothesis driven.

In this study, ROI analyses were used in all the experiments examining neural correlates of number processing. Dehaene and colleagues [50] in a meta-analysis of number processing studies using fMRI identified five key parietal regions involved in number processing. Since the authors attributed specific functions for each of these regions, an ROI analysis in these regions enabled us to better interpret our results and avoid the multiple comparison problem.

For each subject, beta values for each condition were estimated for each ROI by fitting the GLM to the average time course of all the voxels within the ROI. These beta values represent the average percent signal change for a condition and further statistical analyses were performed on these beta values.

Chapter 3 : Parietal Dysfunction during Number Processing in Children with Fetal Alcohol Spectrum Disorders

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Abstract

Number processing deficits are frequently seen in children prenatally exposed to alcohol. Although the parietal lobe, which is known to mediate several key aspects of number processing, has been shown to be structurally impaired in fetal alcohol spectrum disorders (FASD), effects on functional activity in this region during number processing have not previously been investigated. This fMRI study of 49 children examined differences in activation associated with prenatal alcohol exposure in five key parietal regions involved in number processing, using tasks involving simple addition and magnitude comparison. Despite generally similar behavioural performance, in both tasks greater prenatal alcohol exposure was related to less activation in an anterior section of the right horizontal intraparietal sulcus known to mediate mental representation and manipulation of quantity. Children with fetal alcohol syndrome and partial fetal alcohol syndrome appeared to compensate for this deficit by increased activation of the angular gyrus during the magnitude comparison task.

3.1. Introduction

Prenatal alcohol exposure causes impairment in brain structure and function, leading to cognitive and behavioral deficits that range in severity [30, 38, 71, 123]. Fetal alcohol syndrome (FAS), the most severe of the fetal alcohol spectrum disorders (FASD), is characterized by distinctive craniofacial dysmorphology (short palpebral fissures, thin vermillion, flat philtrum), small head circumference and pre- and/or postnatal growth retardation [17]. The craniofacial dysmorphology is also seen in partial FAS (PFAS), together with either small head circumference, retarded growth, or neurobehavioral deficits. Heavily exposed (HE) individuals lacking the distinctive dysmorphology are diagnosed with alcohol related neurodevelopmental disorder (ARND) if they exhibit cognitive and/or behavioral impairment [18].

Prenatal alcohol exposure is associated with a broad range of cognitive deficits, including low IQ [1, 2], poor attention and executive function [6, 7, 124, 125], and slower cognitive processing speed [1, 9-11]. Among the cognitive deficits seen in relation to prenatal alcohol exposure, arithmetic is especially sensitive, and mathematical deficits are seen even after controlling for IQ [1, 2, 12-15], and impaired numerosity is already seen in infants with FAS [126]. When academic

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achievement tests are administered to exposed individuals, arithmetic is consistently more impaired than reading or spelling [127-130].

Although the parietal lobe has been known to be involved in number processing since the beginning of the 20th century [131], fMRI has provided a more extensive understanding of the neuroanatomy of this domain of processing. Based on brain lesion and neuroimaging findings, Dehaene and associates [132, 133] have proposed a triple-code model of number processing that incorporates the three different systems of representation that may be used in number processing tasks: the quantity system, the verbal system and the visual system. In addition, they have posited a core quantity system—a nonverbal abstract representation of numerical quantity, which has been localized bilaterally in the anterior portion of the horizontal segments of the intraparietal sulci (IPS). This area is hypothesized to support number processing irrespective of the notation used; that is, whether represented symbolically as Arabic numbers or sequences of words or analogically by numbers of dots. The verbal processing of numbers is posited to be based in the left angular gyrus (close to the language areas), while the bilateral posterior superior parietal lobules (PSPL) are hypothesized to be involved in spatial and non-spatial attentional processes contributing to the visual processing of numbers. Rivera *et al*. [134] found age-related increases in activation of the anterior IPS during number processing between school age and early adulthood, which they attributed to the development of increasing functional specialization of this region as symbolic number processing becomes increasingly automatic. Results from a meta-analysis of studies comparing number processing in children vs. adults generally support Dehaene's model but suggest that the localization of parietal activations is more notation-specific in children and that right IPS activations in nonsymbolic magnitude comparison are slightly more anterior than those observed in adults [135].

Data from behavioral studies that we have conducted in two different cohorts suggest that a specific deficit in the ability to represent and manipulate quantity may play a critical role in the poor arithmetic performance seen in FASD [16]. A 224-item, 7-subtest, computer-based number processing test that we developed in collaboration with S. Dehaene, was administered to 262 adolescents from the Detroit Longitudinal Prenatal Alcohol Exposure Cohort. A factor analysis of the seven subtests yielded two factors, one reflecting exact and approximate calculation ("Calculation"). The other factor, on which number comparison and proximity judgment loaded most strongly, reflected the ability to represent and manipulate quantity ("Magnitude Comparison"), corresponding to Dehaenes' core quantity system. In a path analytic model, the relation of prenatal alcohol exposure to calculation was fully mediated by its effects on magnitude comparison, suggesting that magnitude comparison is a core deficit involved in the poor arithmetic performance seen in these children. These findings were subsequently confirmed in a sample of school-age children in Cape Town, South Africa [126]. In the Detroit cohort, attention-deficit/hyperactivity disorder (ADHD) was related to poorer performance on all seven number processing subtests, but, by contrast to the pattern seen in the alcohol exposed children, the associations were markedly stronger with calculation than magnitude comparison. Moreover, IQ significantly mediated the effect of ADHD on calculation, suggesting that effects of ADHD on aspects of calculation not specific to the

representation of number, such as attention and executive function, mediate the poorer number processing seen in that disorder.

This behavioral evidence linking prenatal alcohol exposure to impairment in the core quantity system is consistent with evidence from MRI studies reporting alcohol related structural impairment in the parietal region, including disproportionate size reductions in the parietal lobe [23, 30]. A high resolution structural MRI, surface-based image analysis indicated that the brains of alcohol exposed individuals were narrower in the inferior parietal and perisylvian regions [45], and voxel-based morphometry (VBM) analysis revealed gray matter abnormalities that were most prominent in the left perisylvian cortices of the parietal and temporal lobes [38]. In addition, significant cortical thickness excesses were observed in children with FASD in large areas of bilateral temporal, bilateral inferior parietal and right frontal regions [42].

In the first fMRI study of number processing in FASD, adults with and without prenatal alcohol exposure were administered a task involving subtraction from 11 of a series of numbers that appeared on the screen [81]. Exposed individuals with alcohol related dysmorphology exhibited poorer task performance and lower activation in regions known to be associated with arithmetic processing, including right inferior and left superior parietal regions and medial frontal gyrus, compared with controls. However, it was not clear whether the reduced activation in the dysmorphic participants reflected a specific deficit in fronto-parietal function or completion of fewer problems due to the difficulty of the task. Using simpler proximity judgment and single-digit addition problems, we found that children activate the same fronto-parietal regions activated in number processing by adults [136]. Although children with FAS and PFAS performed as well as controls on the simple tasks administered in the scanner, they activated a markedly more diffuse parietal region extending into the angular gyrus, precuneus and posterior cingulate and for, exact addition, also the postcentral gyrus [60]. However, the FAS/PFAS and control groups did not differ significantly in degree of activation in the anterior portion of the IPS and other regions linked by Dehaene and associates to number processing, possibly due to lack of statistical power in the small sample on which that whole brain voxel-wise analysis was conducted.

In this study, we examine the effects of both FASD diagnosis and continuous measures of prenatal alcohol exposure on brain activation in the five parietal structures found by Dehaene and associates to mediate number processing in a larger sample that includes not only children with FAS/PFAS and nonexposed controls, but also nonsyndromal heavily exposed (HE) children [137]. We have found that continuous measures of prenatal alcohol exposure based on a maternal report obtained during pregnancy are often more sensitive in detecting effects of prenatal alcohol exposure than diagnoses based on dysmorphic features in studies using diverse neuroimaging techniques, including tensor-based morphometry [138] and magnetic resonance spectroscopy [139]. Based on the behavioral findings from our Detroit and Cape Town studies, our central hypothesis was that prenatal alcohol exposure would be associated with reduced activation of the anterior IPS, the region believed to mediate abstract representation of numerical quantity.

3.2. Methods

3.2.1. Participants

Participants were 65 right-handed, 8- to 12-year-old children from the Cape Coloured (mixed ancestry) community in Cape Town, South Africa, of whom 40 had been heavily exposed to alcohol prenatally and 25 were controls in the same age range [140]. The Cape Coloured community is composed primarily of descendants of white European settlers, Malaysian slaves, Khoi-San aboriginals, and black African ancestors. The incidence of FASD in this population is exceptionally high due to poor socioeconomic circumstances and historical practices of compensating farm laborers with wine, which have contributed to a tradition of heavy recreational weekend binge drinking [19]. Thirty-seven children were the older siblings of participants in our Cape Town Longitudinal Cohort [141]. The others were identified by screening all of the 8- to 12-year-old children from an elementary school in a rural section of Cape Town, where there is a very high incidence of alcohol abuse among local farm workers [60].

3.2.2. Procedure

Our research nurse and staff driver transported the mother and child from their home to our child development laboratory at the Faculty of Health Sciences campus of the University of Cape Town (UCT) for a 3-hour neuropsychological assessment and to the Cape Universities Brain Imaging Centre (CUBIC) for a neuroimaging assessment, which was administered on the following day. All examiners were blind with regard to maternal alcohol history and the child's FASD diagnostic status, except in the most severe cases where it was obvious. Written informed consent was obtained from each mother; written assent, from each child. Approval for human research was obtained from the Wayne State University Human Investigation Committee and the UCT Faculty of Health Sciences Human Research Ethics Committee.

Each mother was interviewed in her primary language, Afrikaans or English, regarding her alcohol consumption during pregnancy, using a timeline follow-back approach [142, 143]. Volume was recorded for each type of beverage consumed each day, converted to absolute alcohol (AA) using multipliers proposed by Bowman *et al* [144], and averaged to provide summary measures of alcohol consumption during pregnancy. Two groups of women were recruited: (1) heavy drinkers, who consumed at least 14 standard drinks per week (1.0 oz AA⁄day) on average or engaged in binge drinking (5 or more drinks⁄occasion) and (2) controls whose mothers abstained or drank only minimally during pregnancy—92.0% (all but two) of the control mothers abstained; one drank 2 drinks on 11 occasions; the other, 2 drinks on 1 occasion. Data from the alcohol consumption interview were tabulated to provide three continuous measures of drinking during pregnancy: average oz AA consumed per day, AA/drinking day (dose/occasion) and frequency (days/week). Number of cigarettes smoked on a daily basis was also recorded, as was the use of illicit drugs (days/week). Mothers were also interviewed regarding their education. Mothers and children were given breakfast, lunch, and a snack during the morning at each laboratory visit. The mother received a small monetary compensation for each visit and photograph of her child, and the child was given a small gift.

In September 2005, we organized a clinic in which each child was independently examined for growth and FAS dysmorphology by two expert U.S.-based FAS dysmorphologists (H.E. Hoyme and L.K. Robinson) using the Hoyme et al. protocol [5]; a subset of children who could not attend the clinic was examined by a Cape Town-based dysmorphologist (N. Khaole) [141]. There was substantial agreement among the examiners on the assessment of all dysmorphic features, including the three principal fetal alcohol related features—philtrum and vermilion (which were measured on the Astley and Clarren [109] rating scales) and palpebral fissure length (median $r = 0.78$). FAS and PFAS diagnoses were determined at a case conference by the dysmorphologists (HEH and LKR), SWJ, JLJ, and CDM. Nine children met the Hoyme et al. criteria for full FAS: at least two of the principal dysmorphic features, small head circumference (bottom 10^{th} percentile), and low weight or short stature (bottom 10th percentile). Nine met the criteria for PFAS; that is, two features, confirmed maternal alcohol consumption during pregnancy, and at least one of the following: small head circumference, low weight, short stature, or low IQ (<70).

FAS and PFAS groups were combined for the analyses, but HE children were treated as a separate group. The decision not to combine the FAS/PFAS and HE groups was based on our findings in Diwadkar *et al.* [73] that for some aspects of cognition, distinctly different brain activation patterns are seen in these two groups. Analyses were repeated with all alcohol exposed children combined into one group.

3.2.3. Neuropsychological Assessment

Each child was administered the computer-based number processing test described above, which included two 16-item subtests, one of which assessed Exact Addition (EA); the other, Proximity Judgment (PJ) [83, 91]. In EA, a series of problems involving single and⁄or double digits are displayed on the screen, and the child enters the solution on the computer keypad. In PJ, a double-digit number is displayed on the left side of the screen, and the child presses a button to indicate which of two double-digit numbers shown on the right side is numerically closer to it. Each child was also assessed on 7 of the 10 subtests from the Wechsler Intelligence Scale for Children, Third Edition (WISC-III)—Similarities, Arithmetic, Digit Span, Symbol Search, Coding, Block Design, and Picture Completion—and Matrix Reasoning from the WISC-IV. The IQ subtests were selected to represent the four dimensions of the WISC-III: Verbal Comprehension (Similarities), Perceptual Organization (Block Design, Picture Completion, Matrix Reasoning), Freedom from Distractibility (Digit Span, Arithmetic), and Processing Speed (Coding, Symbol Search). Only Similarities were administered in the verbal domain because the other verbal subtests appeared to be less valid in this cross-cultural context. IQ was estimated from these subtests using Sattler's [145] formula for computing Short Form IQ; validity coefficients for Short Form IQ based on 5 or more subtests consistently exceed $r = 0.90$. Handedness was assessed with the Annett (1970) Behavioral Handedness Inventory.

3.2.4. Neuroimaging Assessment

Magnetic Resonance Imaging Protocol. All scans were acquired using a 1.5T Magnetom Symphony MRI scanner (Siemens Medical Systems, Erlangen, Germany). High-resolution anatomical images were acquired in the sagittal plane using a three-dimensional inversion recovery gradient echo sequence (72 slices, TR = 1900 ms, TE = 3.93 ms, TI = 1100 ms, slice thickness 2 mm, 250 mm field of view, resolution 1.4 x 1.0 x 2 mm^3). During the fMRI protocol, 154 functional volumes sensitive to blood oxygen level dependent contrast were acquired with a T2*-weighted gradient echo, echo planar imaging sequence (TR = 2000 ms, TE = 50 ms, 20 interleaved slices, 5 mm thick, gap 1 mm, 230 mm field of view, resolution 3.6 x 3.6 x 5 mm^3).

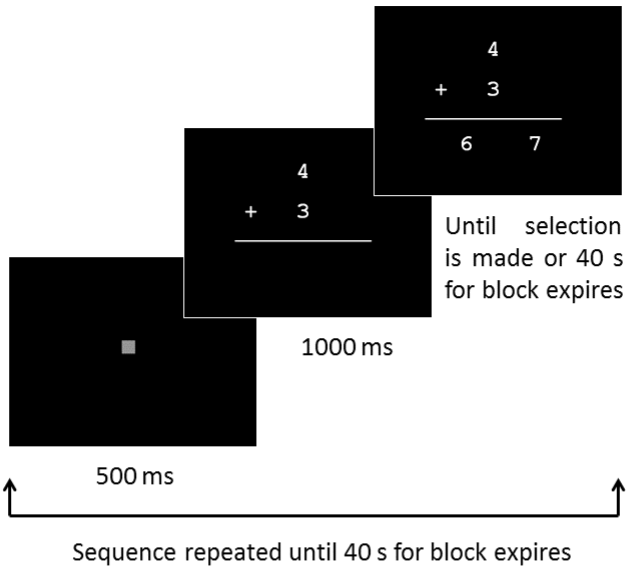
Functional MRI Experimental Tasks. Simplified versions of the EA and PJ subtests from the computer-based number processing assessment described above were administered. The tasks were simplified to make it easier for the children in the exposed groups to achieve acceptable scores for task accuracy in the scanner. EA was simplified in that the child selected the correct answer from two choices displayed on the screen and the sum was never greater than 12. PJ was simplified by including fewer difficult problems (i.e., problems with response choices that were only 1-2 units apart). EA was selected because it is the easiest of the calculation subtests; PJ was selected to represent magnitude comparison because the range of the neurobehavioral scores for Number Comparison is truncated. Each child practiced these tasks initially in a mock scanner built for this study, which was important in reducing anxiety and facilitating completion of the MRI scans. The experimental tasks were programmed using E-Prime software (Psychology Software Tools, Inc., Pittsburgh, PA) and were presented using a data projector and a rear projection screen mounted at the foot of the patient bed. The child held a Lumitouch response system (Photon Control Inc., Burnaby, Canada) in his/her right hand and responded using the right index and middle finger. The child was able to talk to the examiner using an intercom that is built into the scanner and could stop the scan at any time by squeezing a ball held in his⁄her left hand.

The EA and PJ tasks were administered using a self-paced block design, in which the child completed as many problems as possible during each 40 s block. In the active blocks, a fixation square was displayed for 500 ms prior to each trial (Figure 3.1A). In the EA blocks, two numbers were displayed one above the other for 1000 ms, after which two possible solutions appeared horizontally below the two numbers. The display remained on the screen until either the child made a selection or the 40 s time limit for the block expired. Sums were selected randomly from a list in which the solution was never greater than 12 and from which tie problems (e.g., 2 + 2) and sums involving unity had been excluded. The child selected the correct answer from the two choices displayed below by pressing the button on the same side as the correct answer. The control blocks in the EA task (EA_CTL) followed the same format but with two identical Greek symbols displayed vertically initially for 1000 ms (Figure 3.1B). Two different Greek symbols were then displayed horizontally below the vertical symbols and the child selected the one that was identical to the initial two by pressing the button on the side of that symbol. Each block was repeated three times in the following order: EA, EA_CTL, rest, EA, EA_CTL, rest, EA_CTL, EA, rest. In the rest blocks, the fixation square was displayed for 20 s, resulting in a total task

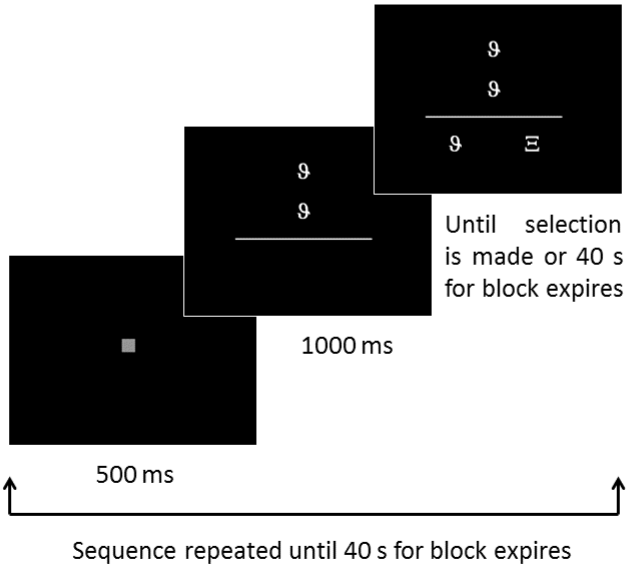
duration of 5 min. The PJ task followed the same format, the only difference being that a single number (the ‘‘target’’) was now displayed for 1000 ms, and the child was instructed to select from two numbers displayed horizontally below it, the one numerically closer to the target. Problems were selected randomly from a list of 1-digit and 2-digit numbers. In the control blocks for the PJ task, the target consisted of a single Greek symbol, followed by two Greek symbols displayed horizontally below it. The child pressed the button on the same side as the symbol that was identical to the target. Each block was repeated three times in the order PJ, PJ control (PJ_CTL), rest, PJ, PJ_CTL, rest, PJ_CTL, PJ, rest, with the fixation square displayed for 20 s during the rest blocks, for a total task duration of 5 minutes.

Behavioral Performance. Responses for the number processing tasks administered in the scanner were recorded on a computer. Number of trials attempted, number of trials correct, and accuracy (% correct) were computed for each task. Functional data were excluded for children with poor task performance (<66% correct) to ensure that all those included in the analysis had been mentally engaged in the neuroimaging task. No imaging data were obtained for 6 children (4 FAS/PFAS, 1 HE, 1 control). For PJ, data of 9 children (3 FAS/PFAS, 3 HE, 3 controls) were excluded from the neuroimaging analyses due to poor performance, and another control child fell asleep during the task. For EA, one HE child was not able to perform the task, and data from 19 children (6 FAS/PFAS, 7 HE, 6 controls) were excluded due to poor performance.

fMRI Analysis. All fMRI analyses were performed using Brain Voyager QX (Brain Innovation, Maastricht, the Netherlands). Four dummy images were acquired in each run that were excluded from all analyses. Images were motion corrected relative to the first volume of the functional run with trilinear/sinc interpolation. Images were corrected for different slice acquisition times and linear trends, spatially smoothed using a Gaussian filter (FWHM 4 mm), and temporally smoothed with a high pass filter of 2 cycles⁄point. All data exceeding movement criteria 3 mm displacement or 3.0° rotation within a functional run were rejected. In addition to the previously noted exclusions for poor performance, PJ data for 2 control children and EA data for 1 control child were discarded due to excessive motion. Each child’s functional data sets were co-registered to his⁄her high-resolution anatomical MRI, rotated into the AC-PC plane and normalized to Talairach space using a linear transform calculated on the anatomical images. The 3.6 x 3.6 x 5 mm^3 fMRI voxels were interpolated during Talairach normalization to 3 x 3 x 3 mm^3.



(A)



(B)

Figure 3.1: Schematic of task design showing the format and timing for blocks of (A) Exact Addition trials and (B) control trials. The same format and timing were used for the Proximity Judgment task.

A priori regions of interest (ROIs) were defined for each of the five parietal regions identified in Dehaene *et al.*'s [50] meta-analysis, namely bilateral anterior horizontal intraparietal sulci (IPS), bilateral PSPL and left angular gyrus. Each ROI consisted of a sphere, radius 6 mm, centered on the coordinates derived from the meta-analysis. These regions are illustrated in Figure 3.2. Separate subject analyses were performed on the average signal in each ROI using the general

linear model with predictors based on the known experimental blocks convolved by the standard hemodynamic function. The six motion correction parameters were z-transformed and then added as predictors of no interest. The beta values generated by this analysis, which reflect the mean percent signal change for each condition for each subject, were used to calculate percent signal change during the numeric task compared to the control task. One outlier with percent signal change values > 3 SD beyond the mean for the right and left PSPL and left IPS regions was excluded from analyses of those regions on the PJ task, and one outlier for the left PSPL and right IPS was excluded from analyses of those regions on EA.

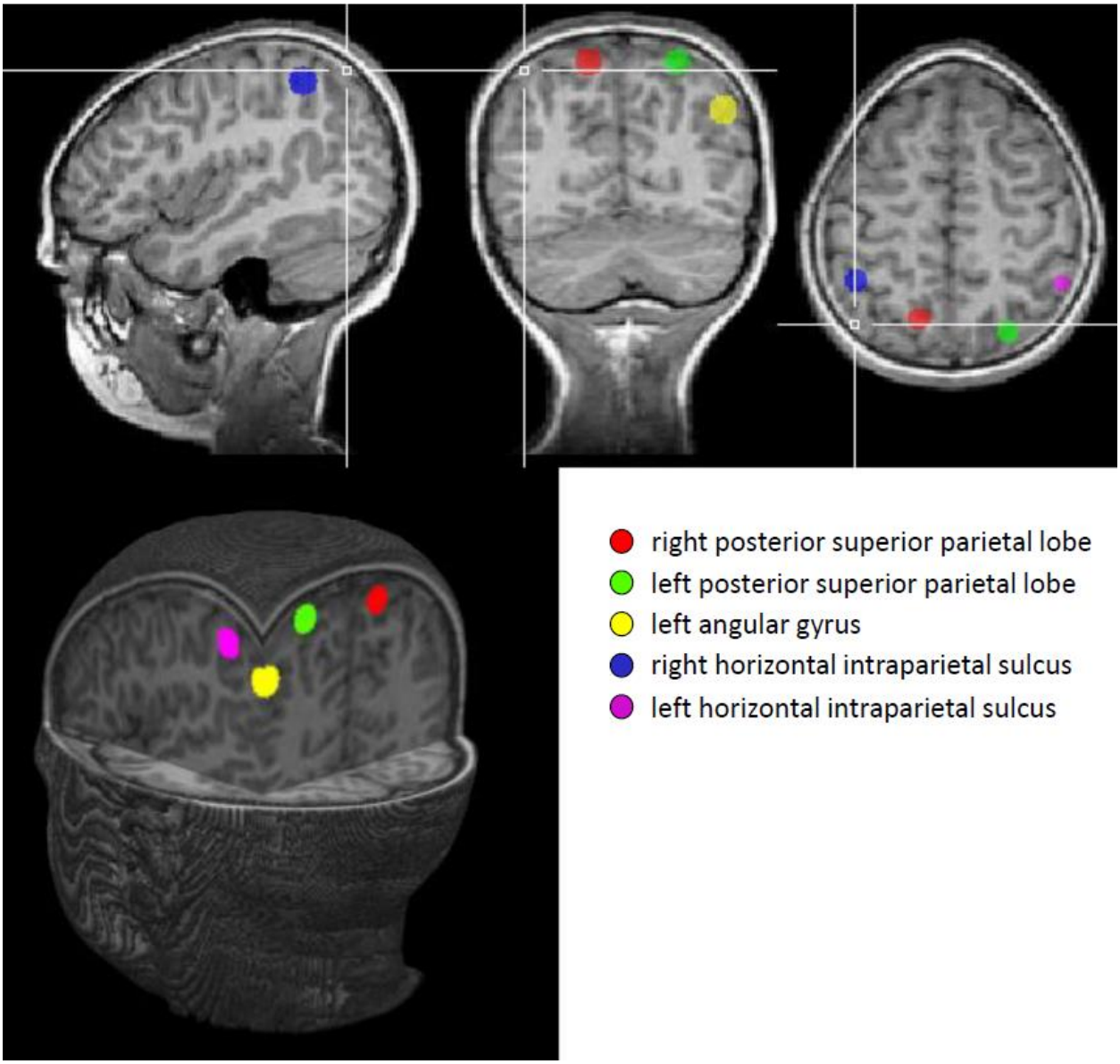


Figure 3.2: Regions identified in Dehaene's meta-analysis that were used as regions of interest in this study.

3.2.5. Statistical Analyses

All variables were examined for normality of distribution. AA/day and AA/occasion were positively skewed and were log transformed (log X +1). The following variables with outliers greater than 3 SD beyond the mean were transformed by

recoding all outlying values to one point beyond the next most extreme observed value: parity (*n*=1), mother's grade (*n*=1), lead exposure (n=1), number of correct EA trials inside the scanner (*n*=1), and PJ accuracy inside the scanner (*n*=2).

Seven control variables were assessed for consideration as potential confounders of the relation of prenatal alcohol exposure to number processing: three demographic characteristics (parity, and mother's age at delivery and years of education), two child characteristics (child sex and age at assessment), and two neurotoxic exposures (maternal smoking during pregnancy and postnatal lead exposure) that are known to impact on the child's academic performance. Lead exposure, which was based on a venous blood sample obtained from the child, was included because lead levels in this population are within the range in which subtle but meaningful effects on cognitive function have been consistently reported (e.g., [15, 146]. Each control variable that was weakly related to a given outcome measure (at $p < 0.10$) was considered a potential confounder of the effect of each exposure measure on the outcome in question.

The outcome measures were accuracy (% correct) inside and outside the scanner, number of trials attempted and number completed inside the scanner, and percent signal change relative to the control task in each of the ROIs for each of the tasks. The relation of diagnostic group (FAS/PFAS; heavily exposed (HE) nonsyndromal; control) to each of the outcome measures was examined using analyses of variance (ANOVA). *Post hoc* comparisons were computed using the least-squares difference (LSD) approach. Each ANOVA was then rerun as an analysis of covariance including as covariates each of the control variables related to the outcome in question at $p < 0.10$ to adjust for potential confounding. The relation of two continuous measures of prenatal alcohol exposure—AA/day and AA/drinking day—to each of the outcome measures was examined using Pearson correlation analysis. Multiple regression analyses were then run relating each of the continuous exposure measures to each of the outcomes controlling for potential confounders.

Pearson correlation was used to examine the relation of percent signal change in each of the ROIs to behavioral performance inside and outside the scanner.

3.3. Results

3.3.1. Sample Characteristics

Sample characteristics for the 65 children recruited are summarized in Table 3.1. The mothers of children with FAS and PFAS reported having consumed an average of 13.2 standard drinks of alcohol per drinking occasion during pregnancy; the mothers of the HE group, 11.8 standard drinks. The groups were generally similar in terms of the control variables, except that the mothers of the HE group smoked more during pregnancy than the mothers of the control or FAS/PFAS children and that on average, the control children's primary caregivers were more educated than either of the other groups. There was very little drug use, with only one mother of an HE child reporting use of marijuana during pregnancy. The FAS/PFAS and HE groups scored more poorly on the WISC IQ test than the control children; and the

FAS/PFAS, more poorly than the HE group. On the WISC subtests, the alcohol exposed children performed more poorly than controls in both the number processing (i.e., Arithmetic) and verbal (i.e., Similarities) domains. The low IQ scores of all of the children in the sample reflect the highly disadvantaged circumstances and poor quality of education available in this community.

Fifty-nine children were scanned: 14 with FAS or PFAS, 21 HE nonsyndromal and 24 controls. After exclusions due to excessive motion and/or poor accuracy, 49 children with usable data for at least one task remained (47 for PJ; 38 for EA). For children included in the analyses, mean displacements did not differ between diagnostic groups ($F(2, 44) = 1.22$, $p = 0.306$ and $F(2, 35) = 0.22$, $p = 0.803$, for PJ and EA, respectively). The children with usable scanner data did not differ from those in the initial sample in terms of alcohol exposure or diagnostic group (all *p*s >0.15). The excluded children were 1.1 years younger on average than those included ($t(63)=3.56$, $p=0.001$), and their estimated IQ scores were lower ($t(63)=2.73$, $p=0.008$), but they did not differ from the children whose data were included in maternal education, parity, smoking during pregnancy , and age at delivery, or child sex and blood lead concentrations (all *p*s>0.10).

3.3.2. Neuropsychological Assessments

Behavioural performance on the number processing tasks is summarized by group and in relation to prenatal alcohol exposure in Tables 3.2 and 3.3, respectively. All the children in the sample are included in the analyses of the data collected outside the scanner, but behavioral performance inside the scanner is shown only for those whose neuroimaging data were included in the data analysis. Outside the scanner the FAS/PFAS group performed more poorly than the other two groups on PJ and more poorly than controls on EA (Table 3.2). Both continuous measures of prenatal alcohol exposure were related to poorer PJ performance outside the scanner, effects that remained significant after controlling for potential confounders (Table 3.3).

By contrast, no group differences in performance accuracy were seen when simplified versions of the tasks were administered inside the scanner (Table 3.2). The only differences between the groups on either task was that in PJ the FAS/PFAS group attempted fewer trials, reflecting a slower rate of completing the PJ problems, and got fewer trials correct than the control group. AA/day and AA/occasion were inversely correlated with number of PJ trials completed correctly and accuracy inside the scanner (Table 3.3).

Table 3.1: Sample characteristics (N=65).

	FAS/PFAS (*n*=18)	Heavy exposed (*n*=22)	Control (*n*=25)	*F* or χ^2
Prenatal alcohol exposure				
Absolute alcohol/day (oz)[a]	2.8 (2.1)	2.1 (1.9)	0.0 (0.0)	19.90***
Absolute alcohol/occasion (oz)[a]	6.5 (3.4)	5.9 (5.4)	0.1 (0.3)	21.98***
Frequency (days/week)[a]	2.9 (1.2)	2.6 (1.8)	0.0 (0.1)	41.29***
Potential confounders				
Maternal				
Parity	2.6 (1.2)	2.0 (1.0)	2.1 (1.4)	1.29
Years of education[b]	6.7 (2.5)	6.9 (2.4)	8.5 (2.0)	4.27*
Smoking during pregnancy (cigs/day)[c]	6.6 (5.1)	12.1 (9.0)	5.6 (7.1)	4.99**
Mother's age at delivery	26.3 (5.9)	24.5 (5.5)	24.0 (5.8)	0.92
Child				
Sex (% male)	33.3	45.5	48	1
Age at assessment	10.1 (1.1)	10.6 (1.3)	10.2 (1.2)	1.09
Blood lead concentration (µg/dl)[d]	7.0 (3.6)	5.2 (1.9)	6.7 (2.6)	2.56†
IQ scores				
WISC Estimated Full Scale IQ score[e]	59.8 (10.5)	67.9 (10.2)	76.0 (11.0)	12.25***
Freedom from Distractibility[f]	74.4 (16.5)	80.6 (10.4)	87.4 (12.4)	5.20**
Arithmetic[g]	4.4 (2.5)	5.9 (2.6)	6.9 (2.6)	4.96**
Digit Span[h]	6.3 (3.7)	7.0 (1.7)	8.3 (2.9)	2.80†
Coding[i]	4.6 (2.3)	6.5 (2.1)	6.8 (2.5)	4.31*
Matrix Reasoning[j]	3.8 (2.1)	4.6 (2.4)	5.4 (2.3)	2.38
Block Design[k]	3.6 (2.1)	5.5 (2.6)	6.4 (3.3)	5.24**
Symbol Search[l]	4.0 (2.2)	4.7 (2.5)	6.8 (2.9)	6.86**
Similarities[m]	2.3 (2.0)	3.7 (2.0)	5.4 (2.4)	11.48***
Picture Completion[n]	4.7 (3.3)	5.2 (3.0)	6.1 (2.9)	1.07

Means (SD). †$p < 0.10$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

[a]cont<FAS/PFAS, HE, both *p*s<0.001

[b]cont > FAS/PFAS, p=0.012; cont > HE, p=0.020

[c]HE > cont, p=0.004; HE > FAS/PFAS, p=0.022

[d]FAS/PFAS > HE, p=0.042; cont > HE, p=0.077

[e]cont > FAS/PFAS, p=0.001; cont > HE, p=0.012; HE > FAS/PFAS, p=0.019

[f]cont > FAS/PFAS, p = 0.002; cont > HE, p = 0.082

[g]cont > FAS/PFAS, p = 0.003; HE > FAS/PFAS, p = 0.067

[h]cont > FAS/PFAS, p = 0.026

[i]Missing for 4 FAS/PFAS, 1 HE, and 4 controls. cont > FAS/PFAS, p = 0.008; HE > FAS/PFAS, p = 0.017

[j]Missing for 2 FAS/PFAS, 1 HE, and 3 controls.

[k]Missing for 1 control. cont > FAS/PFAS, p = 0.002; HE > FAS/PFAS, p = 0.039

[l]cont > FAS/PFAS, p = 0.001; cont > HE, p = 0.009

[m]cont > FAS/PFAS, $p < 0.001$; cont > HE, p = 0.006; HE > FAS/PFAS, p = 0.051

[n]Missing for 1 control.

Table 3.2: Comparison of behavioural performance on Proximity Judgment and Exact Addition by diagnostic group.

	FAS/PFAS	HE	Control	*F*	*F*[a]
Proximity Judgment					
Outside scanner					
N	18	22	25		
Age	10.5 (1.0)	10.6 (1.3)	10.2 (1.2)	1.09	1.09
Accuracy *(% correct)* [b,c,d]	58.0 (29.8)	85.8 (14.3)	88.0(11.8)	14.94****[f]	12.75****
Inside scanner (simplified version)					
N	11	18	18		
Age	10.5 (1.0)	10.9 (1.2)	10.5 (1.2)	0.66	0.66
Trials attempted [b]	33.9 (5.6)	38.9 (8.2)	39.3 (6.1)	2.42†[g]	2.35
Trials correct [b]	29.1 (4.4)	32.9 (7.6)	35.2 (5.2)	3.40*[h]	4.56*
Accuracy (% correct) [b,e]	86.3 (8.6)	84.3 (9.1)	90.1 (9.9)	1.73	2.17
Exact Addition					
Outside scanner					
N	18	22	25		
Age	10.5 (1.0)	10.6 (1.3)	10.2 (1.2)	1.09	1.09
Accuracy (% correct) [b,c]	66.3 (33.8)	81.8 (29.2)	87.8 (19.5)	3.30*[i]	2.63†
Inside scanner (simplified version)					
N	8	13	17		
Age	10.6 (1.0)	11.2 (1.2)	10.5 (1.1)	1.57	1.57
Trials attempted	28.8 (7.7)	34.5 (7.3)	30.4 (8.7)	1.56	1.56
Trials correct [b]	24.4 (7.4)	30.5 (7.6)	27.1 (9.0)	1.47	0.98
Accuracy (% correct) [b,e]	84.7 (12.1)	88.2 (9.8)	88.3 (7.4)	0.45	0.56

Values are means (standard deviation), $^{\dagger}p < 0.10$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$, $^{****}p < 0.001$.

[a]adjusted for potential confounders.

[b]controlled for child's age.

[c]controlled for mother's age.

[d]controlled for lead exposure.

[e]controlled for mother's smoking.

[f]cont, HE > FAS/PFAS, both *p*s<0.001

[g]cont > FAS/PFAS, *p*=0.047; HE > FAS/PFAS, *p*=0.062

[h]cont > FAS/PFAS, *p*=0.012

[i]cont > FAS/PFAS, *p*=0.014; HE > FAS/PFAS, *p*=0.079

Table 3.3: Relation of degree of prenatal alcohol exposure to behavioural performance on Proximity Judgment and Exact Addition.

	N	**absolute alcohol per day** ***r***	**absolute alcohol per day** **ß**	**absolute alcohol per occasion** ***r***	**absolute alcohol per occasion** **ß**
Proximity Judgment					
Outside scanner					
Accuracy *(% correct)* [a,b,c]	65	-0.39****	-0.36***	-0.33**	-0.32***
Inside scanner (simplified version)					
Trials attempted [a]	47	-0.13	-0.12	-0.15	-0.16
Trials correct [a]	47	-0.30*	-0.29*	-0.30*	-0.31*
Accuracy (% correct) [a,d]	47	-0.32*	-0.35*	-0.29*	-0.31†
Exact Addition					
Outside scanner					
Accuracy (% correct) [a,b]	65	-0.22†	-0.18	-0.17	-0.15
Inside scanner (simplified version)					
Trials attempted	38	0.16	0.16	0.1	0.1
Trials correct [a]	38	0.05	0.05	0.03	-0.01
Accuracy (% correct) [a,d]	38	-0.21	-0.37†	-0.15	-0.26

$^{\dagger}p < 0.10$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$, $^{****}p < 0.001$.

[a]controlled for child's age.

[b]controlled for mother's age.

[c]controlled for lead exposure.

[d]controlled for mother's smoking.

3.3.4. Neuroimaging Assessments

The diagnostic groups showed few differences in terms of activation of the five parietal ROIs. On the PJ task, the groups differed only in the left angular gyrus ($F(2,44)=6.93$ $p=0.002$, means ± standard deviation (*M*s ± sd) = 0.17±0.11, -0.02±0.17 and 0.00±0.11 for the FAS/PFAS, HE and control groups, respectively), with more activation in the FAS/PFAS group than in either the HE ($p=0.001$) or control groups ($p=0.003$; see Figure 3.3). On the EA task, none of the ROIs showed significant group differences., but the effect on the right IPS fell just short of significance ($F(2,34)=2.96$, $p=0.065$), with the controls showing more activation than the HE group ($p=0.020$) (*M*s ± sd = 0.03±0.10, -0.03±0.15, and 0.07±0.08 for the FAS/PFAS, HE and control groups, respectively). When combining the FAS/PFAS and HE groups to compare activation in exposed children to unexposed controls, controls showed more activation relative to exposed children in the right IPS during EA ($t(35)=2.06$, $p=0.047$; *M*s ± sd = 0.00±0.13 and 0.07±0.08 for the exposed and control groups, respectively).

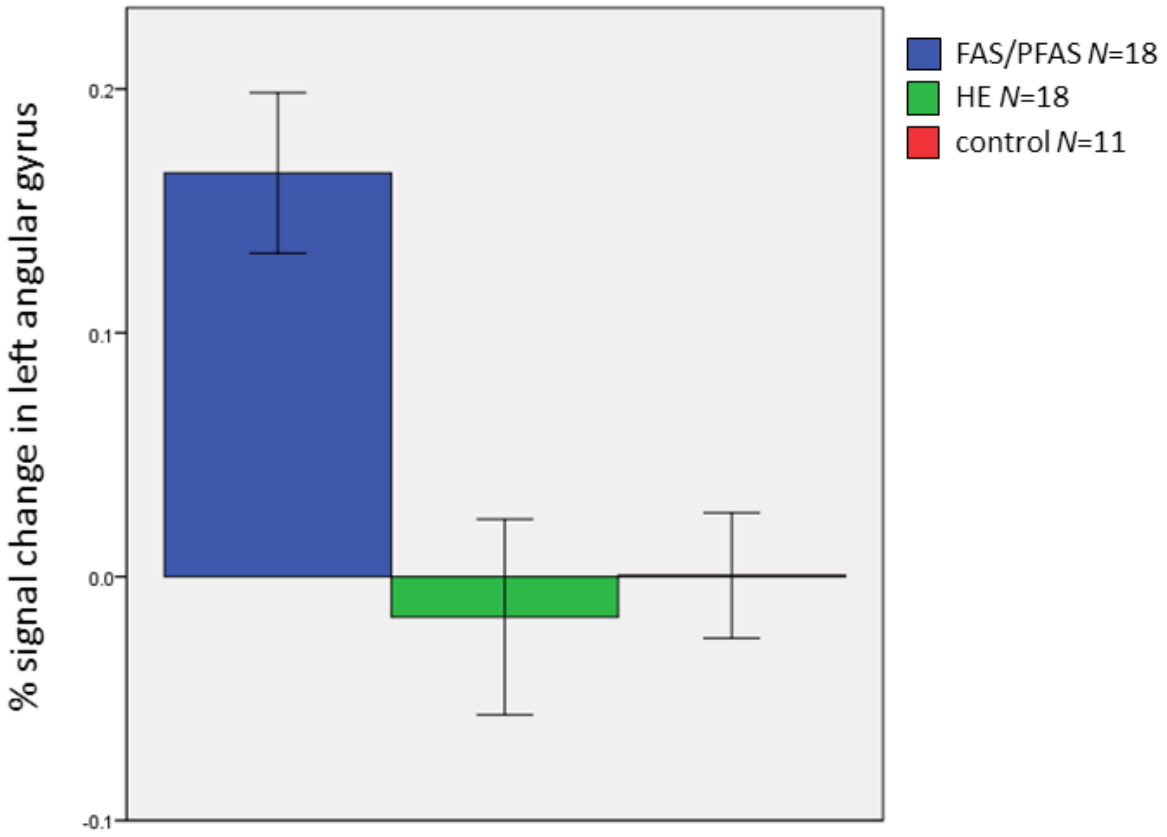


Figure 3.3: Percent signal change during proximity judgment compared to the control task in the left angular gyrus in each of the diagnostic groups (N = 47). Values are means ± standard error.

By contrast, increasing AA/day and AA/drinking day were both related to lower percent signal change relative to the control task in the right IPS on both the PJ and EA tasks (Table 3.4 and Figure 3.4), effects that remained significant after control for potential confounders. Since the children in the FAS/PFAS group performed fewer trials during PJ than the HE and control children, we also examined whether the effects of alcohol on activation during PJ remained significant after controlling for number of trials attempted. All differences and associations remained essentially unchanged (β's relating AA/day and AA/occasion to activation of right IPS were -0.35 and -0.36, respectively; both *p*s < 0.02). In addition, after control for the confounding influences of maternal smoking during pregnancy and age at delivery, greater AA/day was related to reduced activation in the left PSPL during EA, suggesting that these control variables were suppressors. The relation of both continuous measures of prenatal alcohol exposure to reduced percent signal change in the left angular gyrus on the EA task fell short of statistical significance (Table 3.4).

Table 3.4: Relation of extent of prenatal alcohol exposure to the percent signal change compared to a control task in the a priori number processing regions of interest during (a) proximity judgment and (b) exact addition.

(a) Proximity Judgment

	N	Talairach co-ordinates	Absolute alcohol per day r	Absolute alcohol per day ß	Absolute alcohol per occasion r	Absolute alcohol per occasion ß
R posterior superior parietal lobule	46	15,-63,56	0.06	0.06	0.12	0.12
L posterior superior parietal lobule [a]	46	-22,-68,56	-0.08	-0.02	-0.13	-0.06
L angular gyrus	47	-41,-66,36	0.17	0.17	0.18	0.18
R horizontal intraparietal sulcus	47	41,-47,48	-0.36*	-0.36*	-0.37**	-0.37**
L horizontal intraparietal sulcus	46	-44,-48,47	-0.13	-0.13	-0.16	-0.16

(b) Exact Addition

	N	Talairach co-ordinates	Absolute alcohol per day r	Absolute alcohol per day ß	Absolute alcohol per occasion r	Absolute alcohol per occasion ß
R posterior superior parietal lobule [b]	38	15,-63,56	0.25	0.02	0.21	0.01
L posterior superior parietal lobule [c,b]	37	-22,-68,56	-0.12	-0.36*	-0.12	-0.32†
L angular gyrus	38	-41,-66,36	-0.29†	-0.29†	-0.31†	-0.31†
R horizontal intraparietal sulcus [c,d]	37	41,-47,48	-0.48***	-0.49***	-0.43**	-0.46***
L horizontal intraparietal sulcus	38	-44,-48,47	-0.05	-0.05	0.04	0.04

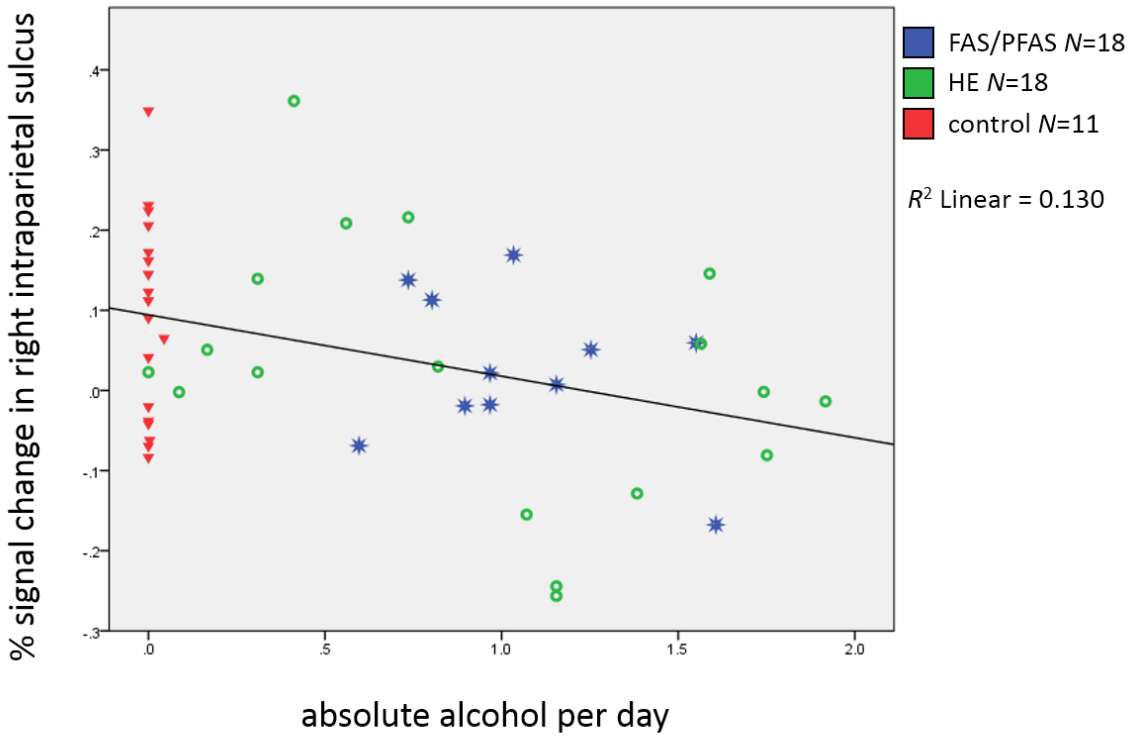
$^{\dagger}p < 0.10$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$.

[a]Controlled for mother's grade.

[b]Controlled for maternal smoking.

[c]Controlled for mother's age.

[d]Controlled for parity.



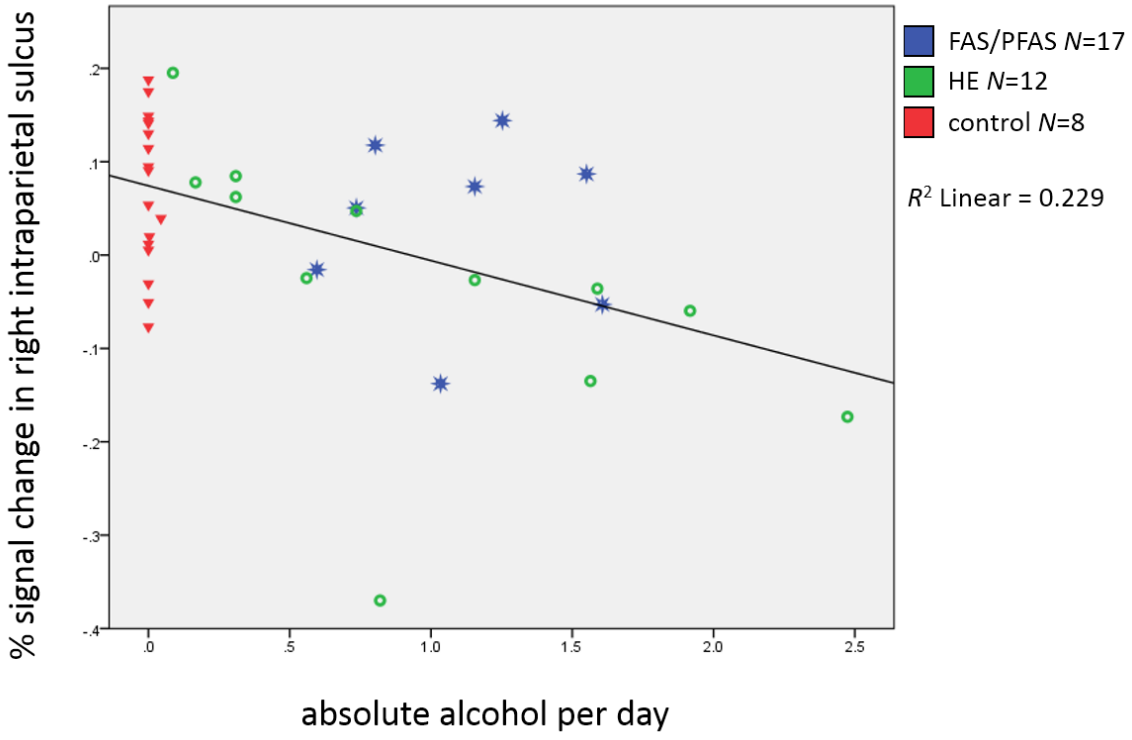


Figure 3.4: Relation of absolute alcohol/day to percent signal change in the right horizontal intraparietal sulcus for PJ (a) and EA (b), respectively.

Greater percent signal change in the right IPS was related to better EA performance outside the scanner (r=0.35, p=0.033). For PJ, greater percent signal change in the right PSPL was associated with poorer accuracy outside the scanner, r=-0.31, p=0.037, and greater signal change in the left PSPL was associated with completion of fewer problems

correctly inside the scanner, r=-0.35, p=0.016. As can be seen in Figure 3.5, the inverse relation between the left PSPL activation and number of correct PJ trials was strongest in the FAS/PFAS group (r = -0.76, p=0.007, for FAS/PFAS, compared with r = -0.40, p = 0.113, for HE and r = -0.31, p = 0.214 for controls).

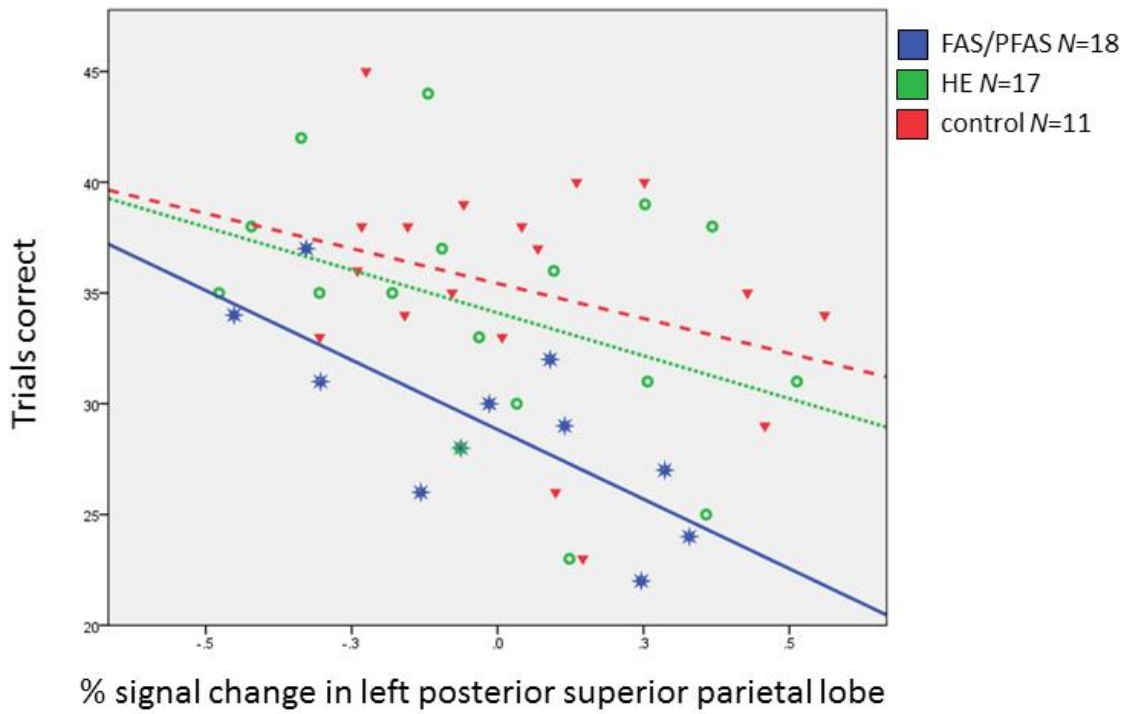


Figure 3.5: Relation of percent signal change in the left posterior superior parietal lobule during proximity judgment to the number of sums answered correctly inside the scanner (FAS/PFAS: R^2 = 0.57; HE: R^2 = 0.16; control: R^2 = 0.10).

3.4. Discussion

This study examined the relation of FASD diagnosis and continuous measures of prenatal alcohol exposure to activation of the five parietal regions identified by Dehaene et al. (2003) as most critical for number processing, using tasks involving simple addition and number comparison. Despite generally similar behavioral performance between diagnostic groups on the simplified tasks administered inside the scanner, the effects of prenatal exposure were observed in altered patterns of brain activation. Greater prenatal alcohol exposure was related to less activation in the right IPS during both the PJ and EA tasks and to less left PSPL activation during EA. In addition, the FAS/PFAS group activated the left angular gyrus more than the other groups in the PJ task.

When more challenging versions of the number processing tasks were administered outside the scanner, FASD diagnosis was associated with poorer performance on both EA and PJ, and degree of prenatal alcohol exposure was associated with poorer performance on PJ. While neither measure of alcohol exposure was related to performance on the simpler

EA task administered in the scanner, the children with FAS or PFAS performed the PJ task more slowly than the other groups in the scanner, and the continuous measures of exposure were associated with less accurate in-scanner PJ performance. These findings suggest that these heavily exposed children found the magnitude comparison processing required for PJ more challenging than simple addition problems that could be solved by rote memory. These findings are also consistent with the higher sensitivity of magnitude comparison to prenatal alcohol exposure seen in our Detroit longitudinal cohort [16].

This study is the first to demonstrate a direct effect of prenatal alcohol exposure on activation of the IPS during number processing. The bilateral IPS have been repeatedly linked to nonverbal representation of quantity [50]. This region is activated when numbers are read even when no arithmetic manipulation is required [147], but activation is greater when comparing numbers [148] or performing calculations [148-150] and often larger on the right [133, 148, 151-153]. This area is more active when manipulating large numbers [154, 155], performing more complex arithmetic manipulations (e.g., with 3 rather than 2 operands [156]), or comparing numbers separated by a small numerical distance [133, 151]. It is well-established that numbers closer together (e.g., 2 and 3) are more difficult (i.e., take longer) to compare than numbers further apart (e.g,. 2 and 9) [157]. Thus, the observed effect of prenatal alcohol exposure on poorer recruitment of the IPS provides evidence of a fetal alcohol related deficit in mental representation and manipulation of quantity, which is consistent with the behavioral evidence from our Detroit study suggesting a specific effect of prenatal alcohol on magnitude comparison [16].

Although our continuous measure of prenatal alcohol exposure showed a dose-dependent decrease in activation of the right IPS during both tasks, diagnostic group differences were *only* seen in this region when all alcohol exposed children were combined into one group and compared to unexposed controls, a result that seems to be driven by the reduced activation in this region during EA in the HE children specifically. This finding is consistent with our previous study, in which children with FAS or PFAS from this cohort were compared with healthy controls using a whole brain, voxelwise approach [60]. Although only the control children showed significant activation of the IPS in that study, the between-group difference was not significant. The only other previous study to examine number processing in relation to fetal alcohol exposure also found an alcohol exposure-dependent response in a right inferior parietal region that included the IPS, with controls showing the most activity, during a subtraction task [81]. The finding that our continuous measures of prenatal alcohol exposure were more sensitive than diagnosis in detecting effects on brain function is consistent with our findings in several other neuroimaging studies [37, 46, 158].

The poorer activation of the right IPS seen in the alcohol exposed children in this study during number processing is also seen in children with developmental dyscalculia (DD) [159-165] and poor arithmetical fluency [166]. DD is a specific learning disability believed to be genetic in origin, which is characterized by impairment in the processing of numerical and arithmetical information in individuals with normal intelligence. In DD, activations of the bilateral IPS also fail to exhibit the increased response to differences in numerical distance seen in normal control children [164]. A voxel-based

morphometry study found less gray matter density in left IPS in low birthweight children with DD, compared with healthy controls [167]. Impaired recruitment of the IPS during tasks involving number processing has also been found in Turner syndrome (TS), a genetic disorder involving a chromosomal defect, in which math is an area where deficits are commonly noted [168]. Similarly, in a study of children with Fragile X syndrome, another form of mental retardation, Rivera *et al* [156] found that, although the right IPS was activated by healthy controls during 3-operand arithmetic problems, it was not activated by Fragile X patients presented with those problems.

In the PJ task, we found greater activation in the left angular gyrus in the FAS/PFAS group than either the HE or control groups. The angular gyrus is adjacent to the perisylvian language processing network and is associated with the verbal processing of numbers [50]. In typically developing children, it is activated more during addition and multiplication than during subtraction, presumably because addition and multiplication facts are more likely to be retrieved from long-term memory [155, 169, 170]. The increased activation of the angular gyrus suggests that children with FAS and PFAS may rely on verbal recitation of the numbers and/or verbally mediated subtraction operations to solve the PJ problems, instead of the type of nonverbal quantity processing that has been shown to be mediated by the right anterior IPS [148]. Verbal mediation may provide a compensatory strategy for these children, whose ability to activate the IPS appears to be impaired. This finding is consistent with results from our previous whole brain voxelwise analysis, in which there was a significant group difference in a the same region of the left angular gyrus [-42,-65,36], with greater activation in the FAS/PFAS group than the controls [60]. We added the HE group to the analyses in the present study and found that, although this group was exposed at similar levels to the FAS/PFAS group, the HE children did not appear to rely on the angular gyrus to perform the PJ task. By contrast, on the EA task, in which verbal recall of number facts is presumed to be the most efficient strategy, our data suggest less activation of the angular gyrus by the more heavily exposed children (Table 3.4).

Lower levels of activation in the right IPS was related to poorer EA performance outside the scanner. By contrast, Price et al. (2013) reported greater activation in the right IPS during single digit arithmetic calculation in adolescents with lower math scores. The authors note that their findings suggest that, while IPS-mediated quantity processing mechanisms appear to play an important role in the development of elementary arithmetic skills, individuals who continue to rely on them in adolescence may achieve poorer mathematical competence than their peers who do not. A large body of behavioral research has shown that children typically undergo a process of development in arithmetic skill whereby simple calculations are initially computed through procedural strategies, but then gradually come to be solved by memory retrieval [171, 172]. Development beyond quantity-based calculation strategies may be essential for the development of more advanced mathematical competence.

In the PJ task, greater activation of the right PSPL was associated with worse performance outside the scanner, and greater activation of the left PSPL was associated with fewer trials correct inside the scanner. The PSPL, which is activated during counting [173] and a variety of visual-spatial tasks, is believed to support the engagement of attention

during number processing [50, 151]. The greater activation of these regions by children with less optimal PJ performance suggests engagement of increased attention to compensate for their poorer facility in magnitude comparison. As shown in Figure 5, the inverse relation between left PSPL activation and PJ performance was strongest in the FAS/PFAS group, which appeared to find the PJ task particularly challenging. By contrast, in the EA task prenatal alcohol exposure was related to less activation of the left PSPL, a region that may facilitate attentional engagement required for simple addition at this age but may no longer be needed by children who have mastered simple proximity judgment.

Since working memory and attention, which are also mediated, in part, by the parietal lobes (e.g., [73, 174]), contribute substantially to number processing performance, impairments in these domains may play a role in the number processing deficits observed in FASD. Moreover, two studies have reported reduced activation in working memory tasks in individuals with FASD compared to controls in posterior parietal regions close to the PSPL ROI used in our study [71, 175]. The specific section of the IPS found to mediate effects of prenatal alcohol exposure on number processing in this study has, however, not been implicated in working memory or attention per se.

One limitation of this study was that the maternal report of drinking during pregnancy was obtained retrospectively several years after the child's birth. Nevertheless, the validity of these reports is supported by our findings that they are predictive of neuroimaging and neurobehavioral outcomes [60, 126, 140]. Predictive validity for childhood IQ was r=-0.36 and -0.40, for AA/day and AA/occasion, respectively, both ps<0.001. A second limitation was the relatively small size of the FAS/PFAS and HE groups and the greater variability in performance accuracy outside the scanner in the FAS/PFAS group. Not surprisingly, the children who were excluded due to poor performance were younger and had lower IQ scores. Presumably, performance on this test will improve with age, and we assume that the fetal alcohol related patterns of brain activation seen in this study will also be manifest once these younger children acquire sufficient math skills to perform these tasks. With regard to IQ, although proportionately more low IQ children were unable to complete the neuroimaging tasks, the sample on whom the neuroimaging data were obtained did include a substantial number of children with low IQs (< 70). Thus, the results can be generalized to all but the most severely retarded children.

Because the children were socioeconomically and educationally deprived, we cannot determine the degree to which the results would hold for children from an educationally less deprived background. Moreover, due to the poor educational background of the children, we had to use very easy problems to ensure that they would be able to perform them. If more difficult problems could have been used, a more pronounced group difference might have been seen. The need to use easy problems also precluded use of a potentially more powerful parametric design to examine the effect of increasing difficulty on the children's brain activations. The age range of the children—7.9 to 13.4 years—was relatively large, particularly in light of the changes in number processing strategy that may emerge during this period. A smaller age range might have yielded stronger associations. Nevertheless, it is interesting, that, although all the behavioural

performance measures were related to age, suggesting an age-dependent effect of education, none of the brain activations were even weakly related to age but instead showed associations with maternal factors (age, education, smoking, and parity). We did not control for multiple comparisons, due to the fact that we examined only a limited number of regions rather than the whole brain. Region-of-interest analyses are advantageous in that multiple comparisons are much less of an issue and SNR is increased by averaging across the voxels in a region.

3.5. Conclusions

This study demonstrates poor recruitment of the right IPS during number processing tasks by children with heavy prenatal alcohol exposure, confirming our previous report based on behavioural data of a fetal alcohol related deficit in mental representation and manipulation of quantity [16]. Our neuroimaging data also indicated increased activation of the angular gyrus in the FAS/PFAS group during the proximity judgment task, suggesting that these children may use a verbal mediation strategy to compensate for impairment in magnitude comparison. By contrast, prenatal alcohol exposure was related to lower levels of activation of the angular gyrus during the exact addition task, for which verbal mediation to retrieve addition facts is presumed to be an efficient strategy. Although activation of the left PSPL, which is believed to support attentional engagement during number processing, was increased in children with less optimal PJ performance, particularly in the FAS/PFAS group, prenatal alcohol was associated with lower levels of activation of this region during the EA task, suggesting less capacity to devote attentional resources to the verbal retrieval of addition facts required for simple addition at this age. In summary, these data demonstrate that prenatal alcohol exposure alters activation of each of the elements the parietal network known to be critical for number processing, providing additional evidence for a specific fetal alcohol related deficit in the core quantity system for representation and manipulation of quantity identified by Dehaene and associates [50]. These findings suggest that remediation focused on the core quantity concepts might be particularly effectively in children with FASD who exhibit difficulty with mathematical processing.

3.6. Acknowledgements

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Chapter 4 : Altered Parietal Activation during Nonsymbolic Number Comparison in Children with Prenatal Alcohol Exposure

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Abstract

The prevalence of fetal alcohol spectrum disorders (FASD) in the Cape Coloured (mixed ancestry) community in the Western Cape Province of South Africa is amongst the highest in the world, providing a unique opportunity to study neural correlates associated with this disorder. Number processing is a cognitive domain particularly affected in FASD and relies on intact parietal functioning. Alcohol-related alterations in brain activation have been found in the parietal lobe during symbolic number processing. However, the effects of prenatal alcohol exposure on the neural correlates of the numerical distance effect and nonsymbolic number comparison have not been investigated. Using functional magnetic resonance imaging (fMRI), we examined differences in brain activation associated with prenatal alcohol exposure in five key parietal regions involved in number processing during a nonsymbolic number comparison task with varying degrees of difficulty.

fMRI data were acquired in 34 Cape Coloured children (8 fetal alcohol syndome (FAS)/partial FAS, 5 heavily exposed nonsydromal, 21 controls; mean age ± SD = 11.55 ± 1.15 years) on a 3T Siemens Allegra in Cape Town, South Africa. Fetal alcohol exposure was assessed by interviewing mothers using a timeline follow-back approach to determine incidence and amount of drinking on a day-by-day basis during pregnancy. Separate subject analyses were performed in Brain Voyager on the average signal in each of five regions of interest, namely bilateral horizontal intraparietal sulci (HIPS), bilateral posterior superior parietal lobules (PSPL), and left angular gyrus (lAG), using the general linear model with predictors of interest for number comparison and difficulty level (the distance effect). Mean percent signal change for each predictor was extracted for each subject for each region to examine group differences and associations with levels of alcohol exposure.

Although groups did not differ in performance, control children activated the right (r) PSPL more during nonsymbolic number comparison than exposed children, and the rHIPS more than syndromal children with FAS/PFAS. These

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impairments appear to be partly compensated for by the lAG, with more heavily exposed children recruiting this region to a greater extent as task difficulty increased (r = 0.46, p < 0.05 for AA/day; AA = oz absolute alcohol). Notably, in nonsyndromal heavily exposed children activation was impaired in the rPSPL, but spared in the rHIPS.

These results extend our previous finding of poor rHIPS recruitment during symbolic number processing, indicating that the mental representation of relative quantity in children is affected by heavy prenatal alcohol exposure for both symbolic and nonsymbolic representations of quantity. Reduced activation of the rPSPL suggests that exposed children may be less able to employ the attentional systems required for optimal number processing.

4.1. Introduction

Prenatal alcohol exposure causes impairment in brain structure and function, leading to cognitive and behavioral deficits that range in severity [30, 38, 71, 123]. The cognitive deficits include low IQ [1, 2], poor attention and executive function [6, 7, 124, 125], and slower cognitive processing speed [1, 9-11]. Among the cognitive deficits seen in relation to prenatal alcohol exposure, arithmetic has been found to be especially sensitive, and mathematical deficits remain, even after controlling for poorer IQ [1, 2, 12-15]. When academic achievement tests are administered to exposed individuals, arithmetic is consistently more impaired than reading or spelling [127-130].

Fetal alcohol syndrome (FAS), the most severe of the fetal alcohol spectrum disorders (FASD), is characterized by distinctive craniofacial dysmorphology (short palpebral fissures, thin upper lip (vermillion), flat philtrum), small head circumference and pre- and/or postnatal growth retardation [17]. In partial FAS (PFAS), some of the craniofacial dysmorphology is seen, as well as either small head circumference, retarded growth, or neurobehavioral deficits. Heavily exposed (HE) individuals lacking the distinctive dysmorphology are diagnosed with alcohol related neurodevelopmental disorder (ARND) if they exhibit cognitive and/or behavioral impairment [18].

The parietal lobe has been known to be involved in number processing since the beginning of the 20th century [131], but recently functional Magnetic Resonance Imaging (fMRI) has provided a more extensive understanding of the neuroanatomy of this domain of processing. Based on brain lesion and neuroimaging findings, Dehaene and associates [132, 133] have proposed a triple-code model of number processing that incorporates three different systems of representation: the quantity system, the verbal system and the visual system. They have posited that the core quantity system—a nonverbal abstract representation of numerical quantity—is localized bilaterally in the anterior portion of the horizontal segment of the intraparietal sulci (IPS). This area is hypothesized to support number processing irrespective of the notation used; that is, whether represented symbolically as Arabic numbers or sequences of words or nonsymbolically by, for example, numbers of dots. The IPS has been shown to be more active when comparing numbers that are closer together (e.g., 2 and 3) than numbers further apart (e.g,. 2 and 9) [151, 164, 176, 177] due to the well-established distance effect [157].

The verbal processing of numbers is posited to be based in the left angular gyrus (close to the language areas), while the bilateral posterior superior parietal lobules (PSPL) are hypothesized to be involved in spatial and nonspatial attentional processes contributing to the visual processing of numbers.

In the first fMRI study of number processing in FASD, adults with and without prenatal alcohol exposure were administered a subtraction task [81]. Exposed individuals with alcohol related dysmorphology exhibited poorer task performance and lower activation in regions known to be associated with arithmetic processing, including right inferior and left superior parietal regions and medial frontal gyrus, compared with controls.

Using proximity judgment (PJ) and single-digit addition problems, children with FAS and PFAS performed as well as controls on simple tasks administered in the scanner, but activated a markedly more diffuse parietal region extending into the precuneus and posterior cingulate and for, exact addition (EA), also into the postcentral gyrus [60]. The FAS/PFAS group exhibited significantly greater activation of the left angular gyrus (AG) than the control children in the PJ task, but no significant differences were detected in the anterior portion of the IPS and other regions linked by Dehaene and associates to number processing, possibly due to lack of statistical power in the small sample on which this whole brain voxel-wise analysis was conducted. In a recent follow on study [82], using the same tasks as the previous study, the effects of both FASD diagnosis and continuous measures of prenatal alcohol exposure on brain activation in the five parietal regions identified by Dehaene *et al*. [50] as most critical for number processing were examined in a larger sample additionally including nonsyndromal heavily exposed (HE) children. During both tasks, greater prenatal alcohol exposure was associated with weaker activation of the right IPS. Additionally, during PJ, children with FAS/PFAS activated the left AG more than control or HE children.

These and other studies examining the effects of prenatal alcohol exposure on neural correlates of number processing have, however, focused on symbolic representation of quantity (e.g. using Arabic numbers or written number words) [12, 16, 60, 81-84]. To date, no neuroimaging studies have examined nonsymbolic number processing (e.g. dot patterns or collections of objects) in alcohol exposed children.

Though abstract representation of number appears to be localized in the IPS in adults [50], a meta-analysis of studies comparing number processing in children vs. adults suggests that the location of parietal activations is more notation specific in children [135]. This, combined with findings of an alcohol related deficit in the core quantity system in the IPS [16, 82], highlights the need to examine the effect of prenatal alcohol on nonsymbolic number processing in children.

Though studies of other conditions associated with mathematical difficulties, such as developmental dyscalculia (DD; characterized by impairment in the processing of numerical and arithmetical information in individuals with normal intelligence) have shown a more pronounced behavioural distance effect in affected children than typically developing controls [163, 178] and poorer maths achievement has been associated with a greater distance effect [179], the distance

effect has been poorly explored in FASD. In DD, activations of the bilateral IPS fail to exhibit the increased response to differences in numerical distance seen in normal control children [164], making this interesting to investigate in FASD.

To the authors' knowledge, the effect of prenatal alcohol exposure on the neural correlates of the numerical distance effect and nonsymbolic number comparison has not been investigated. In this study we used fMRI to investigate the effect of prenatal alcohol exposure on the neural correlates of the numerical distance effect during a nonsymbolic number comparison task. Based on our previous math studies [60, 82] and studies of developmental dyscalculia [163, 164], we hypothesized that prenatal alcohol exposure would be associated with weaker activation in the right IPS during nonsymbolic number comparison, as well as a reduction in the right IPS in activation increases arising from the distance effect. We also expected to find increased compensatory activation in the left AG by children with FASD.

4.2. Methods

4.2.1. Participants

Participants were 34 right-handed children (9.7-13.7 years; median = 11.4 years) from the Cape Coloured (mixed ancestry) community in Cape Town, South Africa, of whom 13 had been heavily exposed to alcohol prenatally and 21 were non-exposed controls in the same age range. The Cape Coloured community is composed primarily of descendants of white European settlers, Malaysian slaves, Khoi-San aboriginals, and black African ancestors. The incidence of FASD in this population is exceptionally high due to poor socioeconomic circumstances and historical practices of compensating farm labor with wine, which have contributed to a tradition of heavy recreational weekend binge drinking [19]. Fifteen children were the older siblings of participants in our Cape Town Longitudinal Cohort [141]. The others were identified by screening all of the 8- to 12-year-old children from an elementary school in a rural section of Cape Town, where there is a very high incidence of alcohol abuse among local farm workers [60].

4.2.2. Procedure

A research nurse and staff driver transported the mother and child from their home to the Cape Universities Brain Imaging Centre (CUBIC) located at the Faculty of Health Sciences campus of Stellenbosch University adjacent to Tygerberg Hospital. All examiners were blind with regard to maternal alcohol history and the child's diagnostic status, except in the most severe cases where it was obvious. Written informed consent was obtained from each mother and assent from each child. Approval for human research was obtained from the Wayne State University Human Investigation Committee and the UCT Faculty of Health Sciences Human Research Ethics Committee.

Each mother was interviewed in her primary language, Afrikaans or English, regarding her alcohol consumption and smoking during pregnancy. The alcohol interviews used a timeline follow-back approach [142, 143] to determine incidence and amount of drinking on a day-by-day basis during pregnancy. Any child whose mother reported consuming at least 14 standard drinks per week (1.0 oz absolute alcohol (AA) ⁄ day) on average or engaged in binge drinking during

pregnancy (4 or more drinks ⁄ occasion) was considered heavily exposed. Controls were children whose mothers reported abstaining or drinking less than 7 drinks per week and no binge drinking during pregnancy. Volumes recorded for each type of beverage consumed each day were converted to absolute alcohol using multipliers proposed by Bowman *et al* [144] to provide three continuous measures of drinking during pregnancy: average oz AA consumed per day, AA/drinking day (dose/occasion) and frequency (days/week). Number of cigarettes smoked on a daily basis was also recorded, as was the use of illicit drugs. Mothers were also interviewed regarding their education and occupational status and that of their spouse/partner and scored for socioeconomic status (SES) using the Hollingshead [180] scale. Mothers and children were given breakfast, lunch, and a snack during the morning at each laboratory visit. At the end of the visits, the mother received a small monetary compensation and photograph of her child, and the child was given a small gift.

Each child was examined for growth and FAS dysmorphology by two expert U.S.-based dysmorphologists during a clinic conducted in 2005; a subset of children who could not attend the clinic was examined by a Cape Town-based dysmorphologist [141]. There was substantial agreement among the examiners on the assessment of all dysmorphic features, including the three principal fetal alcohol related features—philtrum and vermilion measured on the Astley and Clarren [109] rating scales and palpebral fissure length (median $r = 0.78$). Five children met the revised Institute of Medicine criteria [17] for full FAS: at least two of the principal dysmorphic features, small head circumference (bottom 10^{th} percentile), and low weight or short stature (bottom 10th percentile). Three met criteria for PFAS; that is, two features and at least one of the following: small head circumference, low weight, or short stature. Five exposed children did not meet criteria for either FAS or PFAS, and were designated heavily exposed.

4.2.3. Neuropsychological Assessment

Each child was assessed on 7 of the 10 subtests from the Wechsler Intelligence Scale for Children, Third Edition (WISC-III)—Similarities, Arithmetic, Digit Span, Symbol Search, Coding, Block Design, and Picture Completion—and Matrix Reasoning from the WISC-IV. The IQ subtests were selected to represent the 4 dimensions of the WISC-III: Verbal Comprehension (Similarities), Perceptual Organization (Block Design, Picture Completion, Matrix Reasoning), Freedom from Distractibility (Digit Span, Arithmetic), and Processing Speed (Coding, Symbol Search). Only Similarities were administered in the verbal domain because the other verbal subtests appeared to be less valid in this cross-cultural context. IQ was estimated from these subtests using Sattler's [145] formula for computing Short Form IQ; validity coefficients for Short Form IQ based on 5 or more subtests consistently exceed $r = 0.90$. Handedness was assessed with the Annett (1970) Behavioral Handedness Inventory.

4.2.4. Neuroimaging Assessment

Magnetic Resonance Imaging Protocol. All scans were acquired using a 3T Allegra MRI scanner (Siemens Medical Systems, Erlangen, Germany). High-resolution anatomical images were acquired in the sagittal plane using a

magnetization prepared rapid gradient echo (MPRAGE) sequence (TR 2300 ms, TE 3.93 ms, TI 1100 ms, 160 slices, flip angle 12 degrees, 1.3 × 1.0 × 1.0 mm^3, 6.03 min). During the fMRI protocol, 126 functional volumes sensitive to blood oxygen level dependent contrast were acquired with a T2*-weighted gradient echo, echo planar imaging sequence (TR = 2000 ms, TE = 30 ms, 34 interleaved slices, 3 mm thick, gap 0.9 mm, 200 mm field of view, resolution 3.125 x 3.125 x 3 mm^3).

Functional MRI Experimental Tasks. FMRI data were acquired during a nonsymbolic number comparison task, called "Smarties". The child is presented with a screen split in half, each half containing different numbers of smiley faces. The child is required to press the button on the side with the most smiley faces.

Our nonsymbolic number comparison task was administered using a fixed-paced block design and had 9 task blocks, each with 8 problems (16 s), interleaved with 10 s rest blocks. During the task blocks, the children were shown two collections of smiley faces and had to choose the side with more faces. Each task block comprised problems at 1 of 3 levels of difficulty, defined in terms of the ratio of number of faces on one side of the screen to the other (1:2, 2:3, 3:4). There were 3 blocks of each difficulty level, arranged as shown in Figure 4.1. Stimuli were shown for only 1s to prevent counting. During rest blocks the child looked at a fixation square.

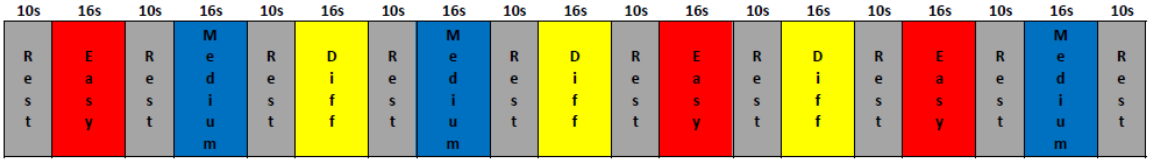

Figure 4.1: Timing diagram of the Smarties task. Easy, medium and difficult (Diff) denote the three difficulty levels, with the ratio of smiley faces on the two sides of the screen being 1:2, 2:3, 3:4 for the three levels, respectively.

Each child practiced this task initially in a mock scanner built for this study, which was important in reducing anxiety and facilitating completion of the MRI scans. The experimental task was programmed using E-Prime software (Psychology Software Tools, Inc., Pittsburgh, PA) and was presented on a rear projection screen using a data projector located in a room behind the scanner through a waveguide in-line with the bore of the magnet. The child held a Lumitouch response system (Photon Control Inc., Burnaby, Canada) in his/her right hand and responded using the right index and middle finger. The child was able to talk to the examiner using an intercom that is built into the scanner and could stop the scan at any time by squeezing a ball held in his/her left hand.

Behavioral Performance. Responses for the Smarties tasks were recorded on a computer; the number of problems correct and the reaction time for correct responses were tabulated. Blocks with fewer than 5 correct responses were modelled as bad blocks. Functional data from children with 5 or more bad blocks were excluded (1 FAS, 1 control). All children met the performance criterion for at least one block at each difficulty level.

The performance criteria were applied to ensure that all children included in the analysis of the neuroimaging data had been mentally engaged in the task.

fMRI Analysis. All fMRI analyses were performed using Brain Voyager QX (Brain Innovation, Maastricht, the Netherlands). Four dummy images were acquired in each run that were excluded from all analyses. Images were motion corrected relative to its first volume with trilinear/sinc interpolation. Images were corrected for different slice acquisition times and linear trends, and temporally smoothed with a high pass filter of 2 cycles/point. For each child, data from the largest section with no movement greater than 3 mm displacement or 3.0° rotation were analysed. Children were excluded from further analyses if the section of usable data did not include at least one block from each condition, resulting in 2 additional children being excluded (1 FAS, 1 control). Each child's functional data were co-registered to his/her high-resolution anatomical MRI, rotated into the AC-PC plane and normalized to Talairach space using a linear transform calculated on the anatomical images. The 3.125 x 3.125 x 3 mm^3 fMRI voxels were interpolated during Talairach normalization to 3 x 3 x 3 mm^3.

A priori regions of interest (ROIs) were defined for each of the five parietal regions identified in Dehaene *et al.*'s [50] meta-analysis, namely bilateral anterior horizontal intraparietal sulci (IPS), bilateral PSPL and left angular gyrus. Each ROI consisted of a sphere, radius 6 mm, centered on the coordinates derived from the meta-analysis. These regions are illustrated in Figure 4.2.

To create a parametric model, difficulty levels were weighted by the ratio of the number of faces on the two sides of the stimuli within each block. We used 2 predictors of interest. The first predictor ("main") gives the activation across all task conditions in contrast to baseline. The other predictor ("parametric") gives the parametric increase in activation across the difficulty levels and is a measure of the strength of the distance effect.

Separate subject analyses were performed on the average signal in each ROI using the general linear model with predictors of interest convolved by the standard hemodynamic function. The six motion correction parameters were z-transformed and then added as predictors of no interest.

For each predictor of interest, beta maps estimating mean percent signal change were created for each subject for each ROI.

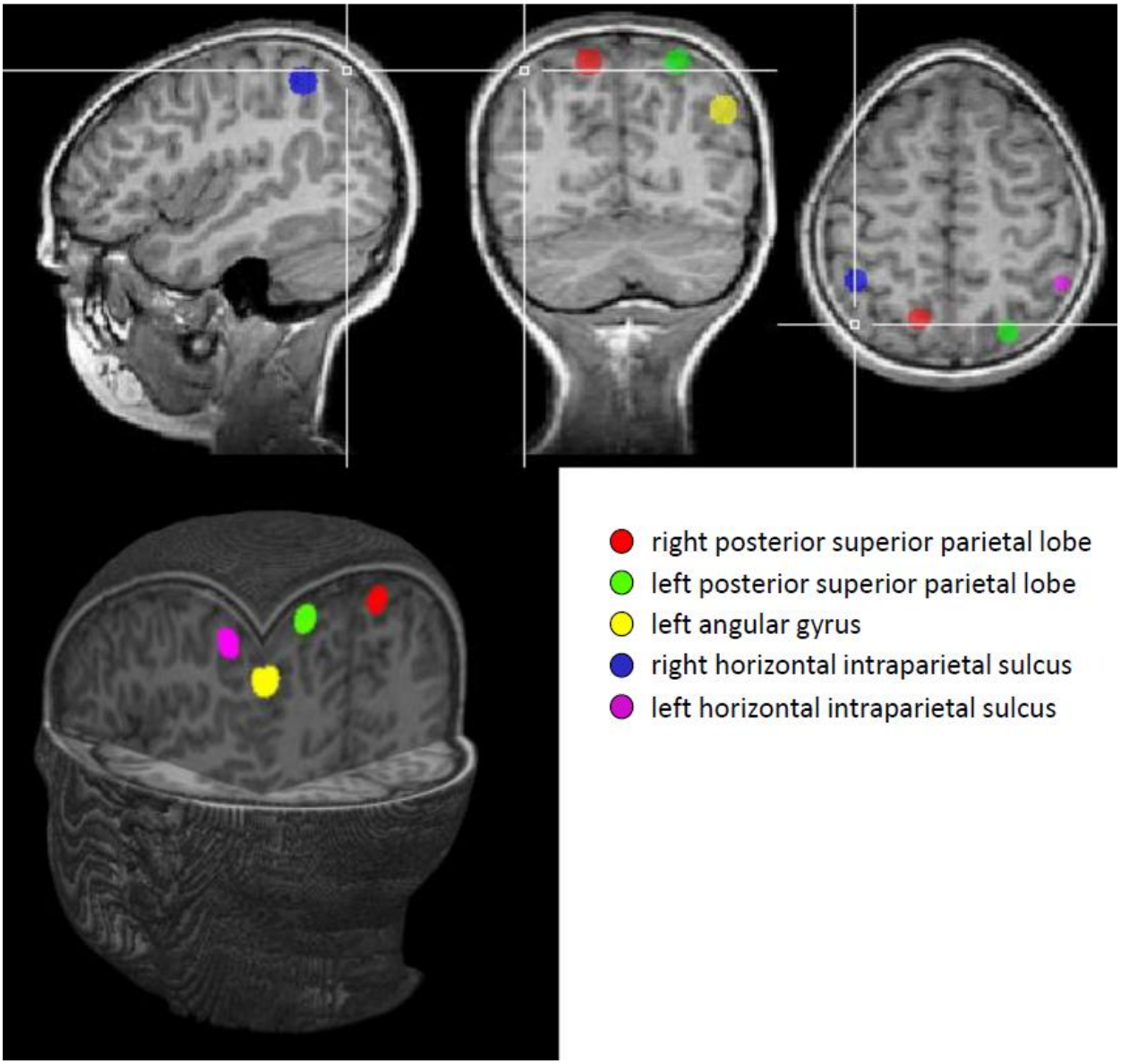

Figure 4.2: Regions identified in Dehaene's meta-analysis that were used as regions of interest in this study.

4.2.5. Statistical Analyses

All variables were examined for normality of distribution. AA/day was skewed and was log transformed (log X + 1). The following variables with outliers greater than 3 standard deviations beyond the mean were transformed by recoding all outlying values to one point beyond the next most extreme observed value: mother's grade (n = 1), smoking during pregnancy (n = 1), AA/occasion (n = 1), and proportional drinking days (n = 1).

Seven control variables were assessed for consideration as potential confounders of the relation of prenatal alcohol exposure to number processing: four demographic characteristics (parity, socioeconomic status, mother's age at delivery, and years of education), two child characteristics (child sex and age at assessment), and one neurotoxic exposure (postnatal lead exposure). Lead exposure, which was based on a venous blood sample obtained from the child, was included because lead levels in this population are within the range in which subtle but meaningful effects on

cognitive function have been consistently reported (e.g., [15, 146]). Each control variable that was weakly related to a given outcome measure (at $p < 0.10$) was considered a potential confounder of the effect of exposure measure on the outcome in question. Although maternal smoking is known to impact on children's academic performance, it was not included as a confounder here as it was strongly related to prenatal alcohol exposure ($r = 0.38$, $p = 0.026$ for AA/day; $r = 0.44$, $p = 0.009$ for AA/occasion, $r = 0.44$, $p = 0.010$ for drinking frequency).

The outcome measures were accuracy (% correct), reaction time (RT) of correct responses, and the "main" and "parametric" activation within each region of interest. Student *t*-tests were used to examine differences in outcome measures between diagnostic groups (exposed; control). Analysis of covariance (ANCOVA), including as covariates each of the control variables weakly related (at $p < 0.10$) to the outcome in question, was used to test whether differences remained significant after controlling for potential confounders. Differences in performance between difficulty levels were examined using a repeated measures ANOVA with Greenhouse-Geisser correction. The relation of the three continuous measures of prenatal alcohol exposure—AA/day, AA/drinking day and proportional drinking days—to each of the outcome measures was examined using Pearson correlation. Multiple regression analyses were then run relating each of the continuous exposure measures and potential confounders to each of the outcomes.

Association of percent signal change in each of the ROIs to behavioral performance was examined using Pearson correlation.

4.3. Results

4.3.1. Sample Characteristics

Sample characteristics for the children are summarized in Table 4.1. The mothers of the alcohol exposed children reported having consumed an average of 13 standard drinks of alcohol per drinking occasion during pregnancy. The groups were generally similar in terms of the control variables, except that the mothers of the exposed group smoked significantly more during pregnancy than the mothers of the control children and that primary caregivers of control children had more years of formal education than the exposed group. No mothers reported using drugs during pregnancy. The exposed group scored more poorly in overall cognitive competence than the control children. The low IQ scores of these children reflect the highly disadvantaged circumstances and poor quality of education available in this community.

Table 4.1: Sample characteristics.

	Exposed (*N*=13)	Control (*N*=21)	*t* or χ^2
Prenatal alcohol exposure			
Absolute alcohol/day (oz)	2.7 (2.3)	0.0 (0.0)	4.19**
Absolute alcohol/occasion (oz)	6.5 (3.3)	0.1 (0.3)	6.96***
Frequency (days/week)	2.8 (1.4)	0.0 (0.1)	7.39***
Potential confounders			
Maternal			
Parity	2.3 (1.1)	2.5 (1.4)	0.47
Years of education	7.0 (2.7)	8.9 (1.8)	2.46*
Smoking during pregnancy (cigs per day)	8.6 (7.9)	2.0 (3.7)	3.32**
Mother's age at delivery	26.5 (5.4)	27.5 (4.4)	0.63
Child			
Sex (% male)	29.4	33.3	0.41
Age at assessment	11.8 (1.2)	11.4 (1.1)	0.87
Blood lead concentration (µg/dl)[a]	6.4 (2.7)	5.8 (2.0)	0.58
Potential mediators			
Estimated IQ score	62.8 (9.3)	76.3 (10.7)	3.75***

Means (SD). †$p < 0.10$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
[a]data only available for 25 subjects

Data from one control child were excluded from all data analyses as responses were not recorded due to a technical error. Two control children were excluded due to incomplete imaging data. Functional data for one exposed and one control child were excluded due to failure to meet the performance criteria, and those of another exposed and control child, due to excessive motion. For the remaining children, mean and maximum displacements did not differ between diagnostic groups ($t(25) = 1.49$, $p = 0.162$ and $t(25) = 1.55$, $p = 0.148$, respectively). Data from 1 control child were excluded from all data analysis as responses were not recorded due to a technical error. Functional data of one child with FAS and 1 control child were excluded due to performance criteria not being met, and that of another child with FAS and another control child due to excessive motion. Additionally, 2 control children were excluded due to incomplete imaging data.

After exclusions, 27 children with usable data remained (11 alcohol exposed and 16 controls). Of the 11 heavily exposed children, 6 had been diagnosed with either FAS or PFAS, while 5 were nonsyndromal. The children with usable scanner data did not differ from those excluded in terms of alcohol exposure or diagnostic group (all *p*s > 0.45), or other demographic variables, including age, IQ, parity, smoking during pregnancy, mother's education, mother's age at delivery, sex or blood lead concentration (all *p*s > 0.112).

4.3.2. Neuropsychological Assessments

Behavioural data are summarised in Table 4.2. Overall accuracy and reaction time did not differ between exposed and control children (all *p*s > 0.255), neither did accuracy and reaction time at each difficulty level (all *p*s > 0.25).

Table 4.2: Comparison of behavioral performance by diagnostic group.

	Exposed (*N*=13)	Control (*N*=20)	*p*
Accuracy (% correct)			
Easy	87.2 (9.4)	89.2 (9.3)	0.555
Intermediate	84.0 (13.5)	86.2 (12.5)	0.623
Difficult	76.0 (14.0)	79.8 (15.3)	0.473
Overall	82.4 (10.5)	85.1 (10.3)	0.47
RT (ms)			
Easy	703.0 (74.5)	671.9 (76.1)	0.256
Intermediate	747.9 (75.7)	718.0 (71.7)	0.261
Difficult	773.2 (109.4)	750.1 (105.3)	0.547
Overall	737.8 (75.4)	708.8 (66.5)	0.255

Values are Means (SD).

None of the behavioural measures was related to alcohol exposure, though the relationship between reaction time in the easy level of the task and AA/day and AA/occasion fell just short of significance (*r*s = 0.30 and 0.34 for AA/day and AA/occasion, respectively, *p*s < 0.095), with greater alcohol exposure being associated with slower reaction time.

Performance accuracy was high in both groups, with scores over 80% for all but the most difficult level. A repeated measures ANOVA with Greenhouse-Geisser correction showed that mean accuracies differed significantly between levels ($F(1.861,59.563) = 12.172$, $p < 0.0005$). Post hoc tests showed that mean accuracy in the easy and intermediate levels were greater than that in the difficult condition ($p < 0.0005$ and $p = 0.001$, respectively). A similar analysis on reaction time showed that mean reaction times also differed between difficulty levels ($F(1.833,58.651) = 14.912$, $p < 0.0005$), with reaction times in the easy condition being less than in the intermediate ($p < 0.0005$) and difficult ($p < 0.0005$) conditions.

4.3.3. Neuroimaging Assessments

Table 4.3 shows the mean % signal change during number comparison in the Dehaene ROIs for children in each of the two groups. The only significant group difference was in the right PSPL, where the control children showed greater activation than the exposed children, who deactivated this region. The left angular gyrus showed a stronger distance effect in the exposed children than the control children (see Table 4.4).

Table 4.3*: Mean percent signal change in the a priori regions of interest during number comparison compared to rest (across all difficulty levels).*

	Talairach coordinates	**Exposed (*N*=11)**	**Control (*N*=16)**	***t***	***F*[a]**
R posterior superior parietal lobule	15,-63,56	-0.2 (0.4)	0.1(0.4)	2.16*	4.69*
L posterior superior parietal lobule [b,c]	-22,-68,56	0.1 (0.5)	0.3 (0.8)	0.78	0.00
L angular gyrus [d]	-41,-66,36	-0.1 (0.3)	-0.2 (0.2)	0.63	0.24
R intraparietal sulcus [c]	41,-47,48	0.0 (0.3)	0.1 (0.3)	1.29	0.80
L intraparietal sulcus [b]	-44,-48,47	0.0 (0.4)	0.1 (0.2)	0.24	0.90

* $p < 0.05$

[a] d.f = (1, 25); adjusted for potential confounders

[b] controlled for maternal education

[c] controlled for child's sex

[d] controlled for child's age

Table 4.4: Mean parametric increase in activation with increasing task difficulty in the a priori regions of interest (parametric effect).

	Talairach coordinates	**Exposed (*N*=11)**	**Control (*N*=16)**	***t***	***F*[a]**
R posterior superior parietal lobule	15,-63,56	-1.0 (1.9)	-0.5 (2.6)	0.49	0.24
L posterior superior parietal lobule	-22,-68,56	-2.4 (3.0)	-1.8 (5.0)	0.39	0.15
L angular gyrus	-41,-66,36	0.7 (1.1)	-0.8 (1.9)	2.42*	5.86*
R intraparietal sulcus [b]	41,-47,48	0.0 (1.3)	-0.2 (1.2)	0.36	0.90
L intraparietal sulcus	-44,-48,47	0.2 (1.0)	-0.7 (2.1)	1.37	1.46

* $p < 0.05$

[a] d.f = (1, 25); adjusted for potential confounders

[b] controlled for maternal education

Since we did not observe a difference in activation during number comparison between exposed and control children in the right IPS as hypothesized, we compared in this region the syndromal FAS/PFAS children *only* to the controls. As hypothesized, the control children activated the right IPS more than the children with FAS/PFAS ($t(20) = 2.14$ $p = 0.045$, means ± SD = -0.15 ± 0.12 and 0.11 ± 0.28 for the FAS/PFAS and control groups, respectively). Notably, when separating the exposed group into children with FAS/PFAS and nonsyndromal HE children, activation patterns in the right PSPL were similar for HE and FAS/PFAS children, while activation patterns in the right IPS of HE children were more similar to those of controls (Figure 4.3).

All three alcohol measures were related to greater distance effects in the left angular gyrus (rs = 0.46, 0.41 and 0.45 for AA/day, AA/occasion and frequency of drinking, respectively, all ps < 0.05). More drinking days per week was related to reduced activation during number comparison in the right PSPL (r = -0.41, p = 0.036).

Children who showed a greater distance effect in the left PSPL (i.e. activated the left PSPL more with increasing task difficulty) had shorter reaction times (r = -0.43, p = 0.03) and those who showed greater activation of this region had better accuracy (r= 0.46, p = 0.02).

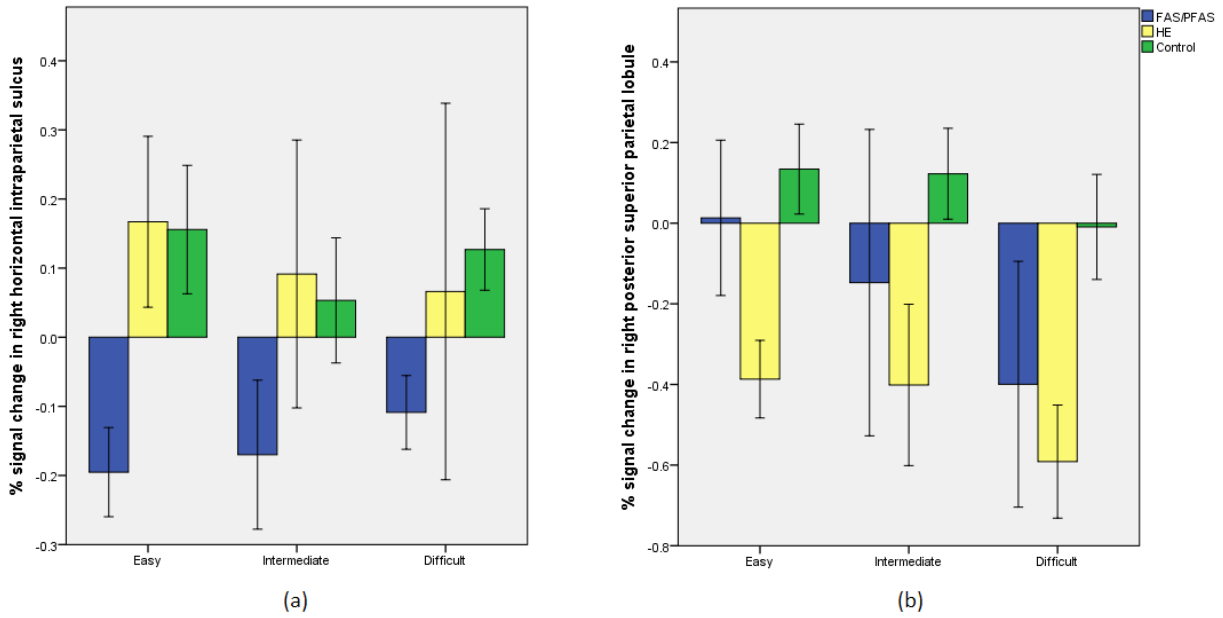

Figure 4.3: Mean percent signal change during each difficulty level of the Smarties task in (a) the right horizontal intraparietal sulcus and (b) the right posterior superior parietal lobule for each of the diagnostic groups. Values are means ± standard error.

4.4. Discussion

This study examined the effects of FASD diagnosis and extent of prenatal alcohol exposure on activation of the five parietal regions identified by Dehaene et al. (2003) as most critical for number processing during a nonsymbolic number comparison task with varying degrees of difficulty. Despite similar behavioral performance, the effects of prenatal exposure could be observed in altered patterns of brain activation. Control children showed greater activation of the right PSPL during nonsymbolic number comparison than exposed children, as well as greater activation of the right IPS compared to the subset of children with a diagnosis of FAS/PFAS. In contrast, the children prenatally exposed to alcohol showed a greater distance effect in the left angular gyrus than control children. In the right PSPL, the activation patterns of HE children were similar to those of the FAS/PFAS children, while in the right IPS their activation patterns were similar to that of the controls. Both greater activation of the left PSPL and a greater distance effect on the activation of this region, were associated with better task performance.

As hypothesized, prenatal alcohol exposure was associated with weaker activation of the right IPS during nonsymbolic number comparison, albeit only in syndromal children. Though this is the first time that prenatal alcohol exposure has been shown to impair the activation of the right IPS during nonsymbolic number processing, this result is consistent with studies of symbolic number processing [60, 81, 82]. During a subtraction task [81], adults with alcohol related dysmorphology showed weaker activation of the right inferior parietal lobe (just slightly superior and posterior to our right IPS ROI) than unexposed controls. In a whole brain analysis [60], we found that control children activated the right IPS more than children with FAS/PFAS during a proximity judgement (PJ) task, though this region was centered more posteriorly than the right IPS ROI used in this study. In our previous study of symbolic number processing using the same *a priori* ROIs, we found that increasing alcohol exposure was related to weaker activation of the same right IPS region used in the current study during both PJ and simple addition [82]. These results suggest that prenatal alcohol exposure impairs the activation of the right IPS during both symbolic and nonsymbolic number processing.

The bilateral IPS have frequently been linked to nonverbal representation of quantity [50]. This region is activated when numbers are attended to, even without any explicit number processing task requirements [147], during number comparison [148] and mental arithmetic [148-150]. The region is independent of modality of number and is activated whether number is presented as Arabic numbers, written number words, spoken numbers or sets of dots [151, 173, 181]. It is more active when manipulating large numbers [154, 155], performing arithmetic with 3 rather than 2 operands [156], approximating addition rather than computing the exact sum [49], or performing subtraction rather than multiplication [148, 182]. This region has also been shown to exhibit a distance effect, meaning that it is activated more when comparing numbers that are closer together [133, 151]. The poorer recruitment of the IPS in children with FAS/PFAS observed here provides further evidence of a fetal alcohol related deficit in mental representation and manipulation of quantity, which is consistent with the behavioral evidence from our Detroit study suggesting a specific effect of prenatal alcohol on magnitude comparison [16].

Based on the findings from developmental dyscalculia [163, 164], we expected to find a weaker distance effect in the right IPS in children with prenatal alcohol exposure, however, this was not the case. Although the control children activated the right IPS more than the children with FAS/PFAS, there was no difference in the distance effect between the groups in this region, and the control children consistently activated the region more than the children with FAS/PFAS across all difficulty levels. The lack of a group difference in the distance effect in this region is likely due to the fact that the distance effect was not really evident in the right IPS in the control children. It is possible that they did not show the distance effect because they are too young and their brains still too immature. In a symbolic number comparison task, adults showed a distance effect in parietal regions, including the intraparietal sulcus [176], while in an identical paradigm, 8 to 12 year old children showed no significant distance effect in any parietal region, even though they showed a behavioural distance effect [183]. An alternative reason may relate to task design. Our "smiley face" stimuli remained the same size throughout trials, so it is possible than children used non-numerical cues, such as density of the faces, or the total area covered by the faces to select the correct answer. Other studies have changed the sizes of their stimuli so that the participants could not use these non-numerical cues to choose the correct answer [179, 184-187]. Another possibility is that the task did not increase enough in difficulty for the additional intraparietal neuronal resources to be required. However, this is unlikely, as the behavioural results did show distance effects. It is also possible that the mechanisms behind the impairment of mathematical ability in prenatal alcohol exposure and developmental dyscalculia are different and that prenatal alcohol exposure does in fact have no effect on the distance effect in the right IPS.

Consistent with our hypothesis, exposed children showed compensatory activation of the left AG, demonstrating a greater distance effect on left AG activation than typically developing controls. This was also evidenced by an association between extent of prenatal alcohol exposure and the distance effect in the left AG. These results suggest that alcohol exposed children need to recruit the left AG to a greater extent as the task difficulty increases, possibly to compensate for deficits in quantity representation in the IPS. In contrast, controls showed a negative distance effect in this region, i.e. reduced left AG activation during the more difficult conditions, presumably due to better functioning of regions specialized for quantity representation. This area has been implicated previously in studies of number processing in FASD. In a whole brain voxelwise analysis, we found a significant group difference in a nearly identical region of the left angular gyrus [-42,-65,36] with greater activation in the FAS/PFAS group than the controls [60]. In a region of interest study, we found that during proximity judgment children with FAS/PFAS demonstrated greater activation of the left AG ROI than HE or control children [82].

The angular gyrus is adjacent to the perisylvian language processing network and is associated with the verbal processing of numbers [50]. It is more activated during addition and multiplication than during subtraction, presumably because addition and multiplication facts are more likely to be retrieved from long-term memory [155, 169, 170]. It is also more active during symbolic number processing than nonsymbolic number processing [188, 189]. Though the left

angular gyrus is usually associated with verbal strategies for solving number processing problems, it is not likely to be the case here, as these nonsymbolic number comparison problems do not involve the recall of arithmetic facts [190], verbal manipulations of number [50], or the mapping from symbols to numerical magnitudes [191, 192].

The left angular gyrus is involved in visuospatial attention [193-198] and has been shown to hold a spatial representation of numbers similar to a mental number line [199]. It is possible that the exposed children rely on this spatial representation increasingly as the difficulty of the problems increase, instead of relying on quantity processing mediated by the right IPS.

It is worth noting that the control and exposed groups showed deactivation of the left AG region compared to baseline for all difficulty levels, except for the difficult condition in exposed children. The fact that the left angular gyrus is part of the default mode network [200] may explain why this region is activated less during the task. Activation changes in this region with difficulty level confirms, however, that it is involved in nonsymbolic number comparison.

Exposed children activated the right PSPL less than control children and more frequent drinking was associated with reduced activation. Though prenatal alcohol exposure has not been shown previously to affect the activation of the right PSPL during number processing, the left PSPL has been implicated. In our previous study, using the same ROIs [82], we found that greater prenatal alcohol exposure was related to less activation of the left PSPL during exact addition. Similarly, a study of subtraction [81] found that a similar region in the left hemisphere was activated more by controls than by exposed young adults with alcohol related dysmorphology.

The PSPL, which is activated during counting [173] and a variety of visual-spatial tasks, is believed to support the engagement of attention during visual processing of numbers [50, 151]. These findings suggest that at this age exposed children seem to be less able to recruit the attentional systems associated with number processing.

It is worth noting that for the exposed group, this region on average is deactivated relative to baseline for all difficulty levels, as well as for the control group for the most difficult level. Though not significant, it appears therefore that children activate this region less as the task becomes more difficult. The default mode network is often deactivated during tasks requiring cognitive processing and shows more deactivation with increasing difficulty of the task [201, 202]. The superior parietal lobule has been found to be part of the default mode network [203], which could explain the pattern of activation found here.

Interestingly, activation patterns in the right PSPL for the nonsyndromal HE children were similar to those of the FAS/PFAS children, while in the right IPS they were similar to controls. It would appear that the activation of right PSPL is impaired, while the functioning of the right IPS is spared in HE children.

The impairment of some regions, but sparing of others in heavily exposed children has been demonstrated previously. A DTI study of heavily exposed children and children with FAS/PFAS showed lower fractional anisotropy in both groups in some regions, while only children with FAS/PFAS were affected in other regions [204]. Similarly, resting-state functional connectivity has been found to be lower in heavily exposed children in only a subset of regions affected in children with FAS/PFAS [205].

Greater activation of the left PSPL, as well as a greater distance effect on the left PSPL, were both associated with better performance. The PSPL can be engaged when attending to specific quantities on the number line [50]. It is possible that the children who are better able to recruit the left PSPL (and recruit it more with increasing difficulty) are better able to position each array of stimuli on the mental number line and therefore to make magnitude comparisons more quickly and accurately.

One limitation of this study was that the maternal report of drinking during pregnancy was obtained retrospectively several years after the child's birth. Nevertheless, the validity of these reports is supported by the fact that they are predictive of neuroimaging and neurobehavioral outcomes [60, 126, 140]. Predictive validity for childhood IQ was r = -0.54 and -0.53, for AA/day and AA/occasion, respectively, both ps < 0.001. A second limitation was the relatively small size of the FAS/PFAS and HE groups. Even though the sample sizes were small, it was worthwhile separating the exposed group into FAS/PFAS and HE, as this enabled us to demonstrate the fact that the HE children's activation of the right PSPL was impaired, while their ability to use the right IPS was spared. Because the children were socioeconomically and educationally deprived, we cannot determine the degree to which the results would hold for children from an educationally less deprived background. In this study, we used a block design. Had we used an event-related design with problems of different difficulty levels randomly presented, we might have been better able to detect parametric effects.

We did not control for multiple comparisons, due to the fact that we examined only a limited number of regions rather than the whole brain. Region-of-interest analyses are advantageous in that multiple comparisons are much less of an issue and SNR is increased by averaging across the voxels in a region. In our task design, the size of the stimuli remained the same size throughout the trials, so it is possible that the children could use non-numerical cues, such as luminance, or the total area covered by the faces to select the correct answer. However, even if the children did use non-numerical cues, it would still involve some type of a magnitude judgment. Though there is some conflicting evidence, it is likely that numbers and other non-numerical magnitudes, including area and luminance, share, at least partly the same magnitude representation system [206].

4.5. Conclusions

This study found poor recruitment of the right IPS during nonsymbolic number comparison in syndromal children with FAS/PFAS, extending the previous finding of poor right IPS recruitment during symbolic number processing [82]. These

results provide convincing evidence that heavy prenatal alcohol exposure impairs mental representation and manipulation of quantity for both symbolic and nonsymbolic representations. As hypothesized, this impairment was compensated for by the left AG, with only exposed children needing to recruit the left AG to a greater extent as the task difficulty increased, and more so as exposure levels increased. A dose-dependent reduction in the activation of the right PSPL in children with prenatal alcohol exposure suggests that alcohol impairs the ability of exposed children to employ the attentional systems required for optimal number processing. Notably, the nonsyndromal HE children's activation was impaired in the right PSPL, but spared in the right IPS.

Chapter 5 : Prenatal Alcohol Related Reductions in Brain Function during Place Learning Are Evident in Boys Only

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Abstract

Although performance deficits in place learning in virtual environments have been reported in fetal alcohol spectrum disorders (FASD), the neural correlates have not been investigated. This functional magnetic resonance imaging (fMRI) study of 57 Cape Coloured children (41 alcohol exposed; 16 controls; mean age ± SD = 9.44 ± 0.42 years; 29 boys) who form part of our longitudinal cohort recruited from a community in which the prevalence of FASD is among the highest in the world, examined the effects of prenatal alcohol exposure (PAE) on the neural correlates of place learning in a virtual reality Computer-Generated (CG) Arena. Timeline follow-back interviews were conducted during pregnancy to assess the mother's alcohol consumption. fMRI data were acquired on a 3T Allegra while children passively viewed a recording of an experimenter completing the CG Arena task. During visible-target blocks, the child observed navigation to a visible gray platform. During invisible-target blocks, the platform only appeared when the experimenter moved over it. All children performed a post-test immediately after the scan during which they had to navigate to the location of the invisible platform. Whole brain group analyses were performed in Brain Voyager using the general linear model with predictors based on known experimental blocks convolved by the standard hemodynamic function. Although there were no group differences in performance (for sex or FASD diagnosis), greater PAE in boys *only* was associated with poorer performance (r=0.38 for path length, r=0.50 for latency, ps<0.044), as well as reduced activation in the parahippocampal gyrus (PHG), precuneus, posterior cingulate, frontal and temporal lobes, caudate, insula, claustrum, lentiform nucleus and thalamus. In girls, PAE was not associated with performance or activation in any regions. Girls and boys are known to use different navigation strategies, with boys relying more on compass directions and gradients ("allocentric navigation"), and girls relying more on landmarks. Poorer recruitment of the PHG, a region that has been shown to mediate allocentric navigation, in boys exposed to higher levels of alcohol may explain the observed dose-dependent place learning deficit. The absence of PAE effects in girls provides further evidence that the landmark strategy may be less affected by PAE.

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5.1. Introduction

Prenatal alcohol exposure (PAE) is associated with impairment in brain structure and function that may lead to cognitive, behavioral, and neurological deficits of variable severity [30, 37, 38, 71, 123]. Fetal alcohol syndrome (FAS), the most severe of the fetal alcohol spectrum disorders (FASD), is characterized by small head circumference, pre- and/or postnatal growth retardation, and characteristic facial features, including short palpebral fissures, thin vermillion and flat philtrum [17]. Two of the three facial features are also seen in partial FAS (PFAS), together with either small head circumference, retarded growth, or neurobehavioral deficits. Heavily exposed (HE) individuals lacking the distinctive pattern of FAS dysmorphology are diagnosed with alcohol-related neurodevelopmental disorder (ARND) if they exhibit cognitive and/or behavioral impairment [17, 18].

Cognitive deficits associated with PAE include lower IQ [1, 2], poor attention and executive function [6, 7, 124, 125, 207], impaired learning and memory [4, 208-211], arithmetic difficulties [1, 2, 12-15, 82], slower cognitive processing speed [1, 9-11], and compromised visual-spatial ability [212-214].

Two distinct components of spatial navigation, spatial location memory and place learning, are both also affected by PAE. Impairment in spatial location memory has been demonstrated using table top tests [92-94], the Visual Learning task from the Wide Range Assessment of Memory and Learning (WRAML) [4, 95, 99], and spatial *n*-back tasks [77-79]. PAE-related place learning deficits have been shown in rodents and in humans. In the Morris water maze (MWM; [215]), ethanol-exposed rodents took longer than unexposed controls to learn escape routes [100-102]. In a computer-simulated version of the MWM [105], boys with prenatal alcohol exposure travelled further than unexposed controls to reach the hidden platform [61]. Children prenatally exposed to alcohol performed worse during a probe trial [85], and travelled a greater distance to reach a hidden platform [216].

Functional MRI (fMRI) and positron emission tomography (PET) studies have identified the hippocampus and parahippocampal gyrus, precuneus, retrosplenial cortex, posterior and inferior parietal cortices, intraparietal sulcus, fusiform gyrus, lingual gyrus, caudate nucleus, thalamus, prefrontal areas, and cerebellum as key regions for navigation [217-231]. Although the hippocampus appears to be essential for successful place learning in the MWM in rodents [232-234] and the virtual versions of the MWM and other virtual environments in humans [235-237], the parahippocampal gyrus, posterior parietal regions (especially the precuneus), fusiform gyrus, and thalamus [238, 239] are also involved.

Sex differences in spatial navigation have been widely reported, with males performing better across a range of ages, including young adulthood [105, 240-247], older adulthood [244], pre-pubertal childhood [248], and adolescence [241]. Notably, some fMRI studies have shown differences in brain activation between males and females during spatial navigation [226, 249], even in the absence of performance differences [250], while others involving passive learning have not [251, 252]. In those that found sex differences, females activated the right inferior parietal lobule, right superior parietal lobule, left superior frontal and right medial frontal gyri, and the right prefrontal cortex more than

males [226, 249], while males showed greater activation of the right and left parahippocampal gyri, left hippocampus and left posterior cingulate [226, 249]. These sex differences in performance and patterns of brain activation suggest that males and females use different navigation strategies.

Notably, in rats alcohol-related deficits were evident only in males [253]. Alcohol-related deficits in place learning were also observed in the only previous human study, which only examined boys [61]. These findings, combined with the known sex differences in strategy and brain activation during navigation, suggest that navigation in males and females may be differentially affected by PAE.

To our knowledge, this is the first study to examine effects of PAE on neural correlates of place learning. This study includes children with FAS and PFAS as well as nonsyndromal HE children. It also examines the association of navigation performance to brain activation during navigation with continuous measures of PAE and investigates whether there are sex differences in the effects of PAE on performance and brain activation during navigation. We hypothesized that alcohol-exposed children would perform more poorly than controls and would also show altered patterns of brain activation. Further, we predicted that boys would perform better than girls on this task, and that there would be sex differences in alcohol-related alterations in brain activation during navigation.

5.2. Methods

5.2.1. Participants

Participants were 57 right-handed 8- to 10-year-old children from the Cape Coloured (mixed ancestry) community in Cape Town, South Africa. Forty-one of these children had been heavily exposed to alcohol prenatally [141]. The Cape Coloured community is composed primarily of descendants of white European settlers, Malaysian slaves, Khoi-San aboriginals, and black African ancestors. The incidence of FASD in this population is exceptionally high due to poor socioeconomic circumstances and historical practices of compensating farm laborers with wine, both of which have contributed to a tradition of heavy recreational weekend binge drinking [19, 254].

5.2.2. Procedure

The children's mothers were recruited while pregnant between 1999 and 2002 at their first visit to an antenatal clinic. Each mother was interviewed, using a timeline follow-back approach [141-143], regarding her alcohol consumption during pregnancy. At recruitment, the mother was interviewed regarding the incidence and amount of her drinking on a day-by-day basis during a typical 2-week period at time of conception. She was also asked whether her drinking had changed since conception; if so, when the change had occurred, and how much she had drunk on a day-by-day basis during the preceding 2-week period. This procedure was repeated in mid-pregnancy and again at 1 month postpartum to provide information about drinking during the latter part of pregnancy. Volume was recorded for each type of beverage consumed each day, converted to ounces of absolute alcohol (AA) using multipliers proposed by Bowman et

al. [144], and averaged to provide three continuous measures of alcohol consumption at conception and during pregnancy: average ounces of AA consumed/day, AA/occasion and frequency of drinking (days/week).

Two groups of women were recruited: (1) heavy drinkers, who consumed at least 14 standard drinks per week (1.0 oz AA⁄day) on average or who engaged in binge drinking (5 or more drinks⁄occasion), and (2) controls whose mothers abstained or drank no more than minimally during pregnancy. Number of cigarettes smoked/day was also recorded, as was the use of illicit drugs (days/week). Mothers were also interviewed regarding their age at delivery, education (years completed), and marital status.

In September 2005, we organized a clinic in which each child was independently examined for growth and FAS dysmorphology by two expert FAS dysmorphologists (H.E. Hoyme, MD, and L.K. Robinson, MD) using the Hoyme et al. [17] protocol [141]. A subset of children who could not attend the clinic was examined by another FAS dysmorphologist (N. Khaole, MD). There was substantial agreement among the examiners on the assessment of all dysmorphic features, including the three principal fetal alcohol related features—philtrum and vermilion (which were measured on the Astley and Clarren [109] rating scales) and palpebral fissure length (median r = 0.78). FAS and PFAS diagnoses were agreed upon at a case conference by the dysmorphologists (HEH and LKR), SWJ, JLJ, and CDM. Eight children met the Hoyme et al. criteria for full FAS and 19 for PFAS. The 14 alcohol exposed children who did not meet criteria for either FAS or PFAS were designated nonsyndromal HE.

Written informed consent was obtained from each mother; written assent, from each child. Approval for human research was obtained from the Wayne State University and UCT Faculty of Health Sciences Human Research Ethics Committees.

5.2.3. Neuropsychological Assessment

IQ data were collected from the children on the Wechsler Intelligence Scale for Children-IV (WISC-IV) at 10 years [73, 140]. At the 5-year follow-up of these children, we administered the Junior South African Individual Scales (JSAIS; [255]), which are available in Afrikaans and in English and have been normed for South African children. IQ scores from the JSAIS were strongly correlated with the WISC-IV scores for the children in this sample, r = 0.74, $p < 0.001$, supporting the validity of the WISC for use with this population.

5.2.4. Neuroimaging Assessment

Magnetic Resonance Imaging Protocol. All scans were acquired using a 3T Allegra MRI scanner (Siemens Medical Systems, Erlangen, Germany). High-resolution anatomical images were acquired in the sagittal plane using a three-dimensional magnetization prepared rapid gradient echo sequence (160 slices, TR = 2300 ms, TE = 3.93 ms, TI = 1100 ms, slice thickness 1 mm, resolution 1.3 x 1.0 x 1.0 mm^3). During the fMRI protocol, 213 functional volumes sensitive to blood oxygen level dependent contrast were acquired with a T2*-weighted gradient echo, echo planar imaging

sequence (TR = 2000 ms, TE = 30 ms, 34 interleaved slices, 3 mm thick, gap 1.5 mm, 200 mm x 200 mm field of view, resolution 3.125 x 3.125 x 3 mm^3).

Functional MRI Experimental Tasks. We used a virtual navigation environment known as the Computer-Generated (CG) Arena [106-108], which presents tasks similar in form and appearance to those presented by the virtual water maze task used in the Hamilton [61] FAS study. All participants practised the task using a desktop-based version of the CG Arena before scanning, and also listened to a recording of the scanner noises while lying in a mock scanner. During the scan, the CG Arena [106, 108] was presented using a data projector positioned in a room behind the scanner. Images were projected through a waveguide in line with the bore of the magnet onto a rear projection screen mounted behind the scanner, which the children viewed using the standard mirror system that mounts to the head coil. Auditory tones were presented using the standard Siemens headphones. The children were able to talk to the examiner using an intercom that is built into the scanner. Children could stop the scan at any time by squeezing a ball held in the left hand.

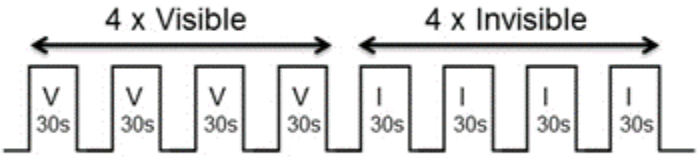

Figure 5.1: Timing diagram of the CG Arena task.

Children were scanned while passively viewing a recording of another person navigating around the CG Arena room to the platform, which was sometimes visible (visible-target condition) and sometimes hidden (invisible-target condition). The task comprised four repetitions of the visible condition (duration 30s each), followed by four repetitions of the invisible condition (duration 30s each), with 21s rest blocks between each active block (Fig 5.1). An initial 19s rest block preceded the first visible block, and a final 20s rest block followed the last invisible block (total task duration 426s). During rest blocks, a static picture of a waterfall was displayed (Fig 5.2(a)). During visible-target blocks, a gray platform was visible on the floor of a circular brick enclosure in a square room (Fig 5.2(b)). To prevent subjects from learning the room during visible conditions, there were no pictures on the walls and the location of the platform changed for each repetition of the visible block. During invisible-target blocks, the platform was hidden (Fig 5.2(c)) until the person navigating moved over it (Fig 5.2(d)). The location of the platform remained the same in each repetition of the invisible condition. Hence, the aim was for the subject to learn the location of the invisible platform using distal cues within the room, such as pictures on the walls.

Behavioral Performance. After completing the scan, the children performed a post-scanner test in a small room adjacent to the scanner to confirm that they had learnt the location of the platform during the invisible-target condition. This test involved using a joystick to navigate within the CG Arena to that location. The test comprised two trials; during each, the subject had 120s to find the platform. Time to navigate to the location of the platform ("latency") and length of path to that location were used to assess task performance. Because the post-scanner assessment indicated that all of the participants had been attending to the task, none of the children were excluded based on performance.

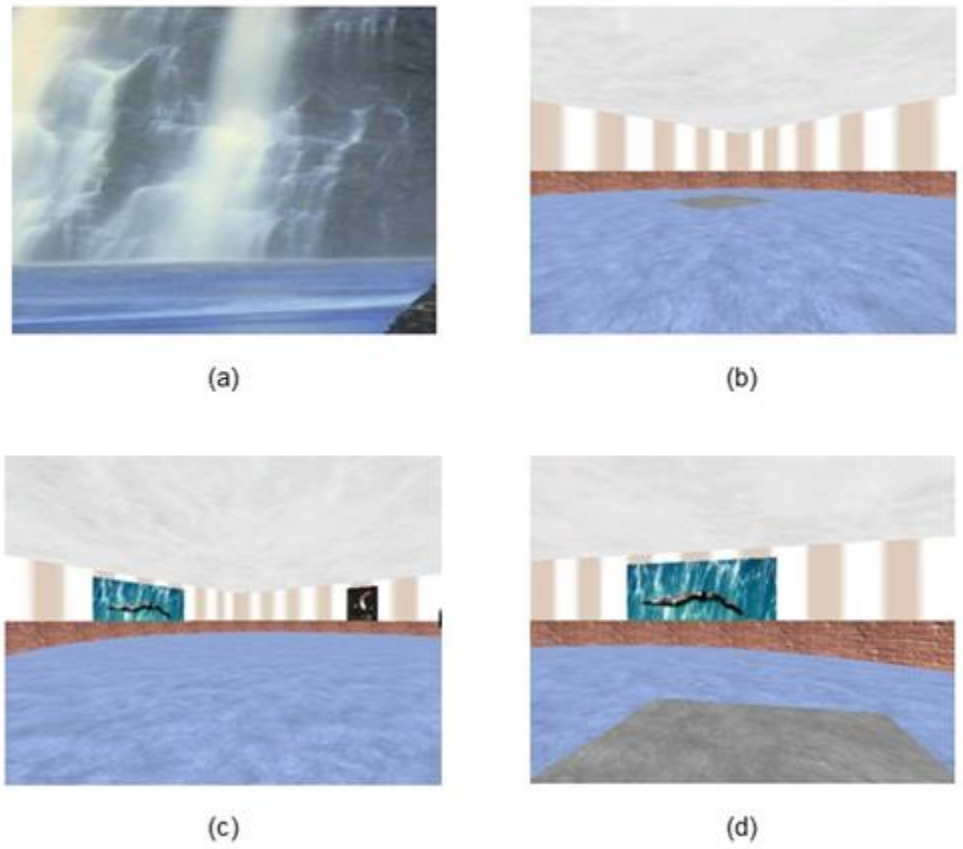

Figure 5.2: (a) picture displayed during rest blocks, (b) example of a visible platform in a room with no pictures, (c) example of a room with an invisible platform, (d) the platform appears when the subject moves over it.

fMRI Analysis. All fMRI analyses were performed using Brain Voyager QX (Brain Innovation, Maastricht, the Netherlands). Four dummy images were acquired that were excluded from all analyses. Images were motion corrected relative to the first volume with trilinear/sinc interpolation. Images were corrected for different slice acquisition times and linear trends and temporally smoothed with a high pass filter of 2 cycles⁄point.

Because the present data were acquired near the end of the imaging session, the data contained substantial motion artefacts. For each subject, data from the largest continuous section with no movement greater than 3 mm displacement or 3.0° rotation were analysed. Children were only included in the analysis if they had usable data from at least one block for each condition.

Each child's functional data were co-registered to his⁄her high-resolution anatomical MRI, rotated into the AC-PC plane and normalized to Talairach space using a linear transform calculated on the anatomical images. The 3.125 x 3.125 x 3 mm^3 fMRI voxels were interpolated during Talairach normalization to 3 x 3 x 3 mm^3.

Whole brain group analyses were performed with a random effect analysis of variance (ANOVA) using a general linear model (GLM) with predictors based on the visible-target and invisible-target experimental blocks convolved by the standard hemodynamic function. The six motion correction parameters were *z*-transformed and added as predictors of no interest. Beta maps were created for each subject for each condition of interest (i.e., visible-target and invisible-target). To examine whether the differential activation between the invisible-target and visible-target conditions differed by sex, the beta maps were analysed at the second level using a repeated-measures ANOVA, with one within-subjects factor (i.e., invisible-target vs visible-target) and one between-subjects factor (i.e., sex). The voxelwise threshold was set to $p < 0.01$, with cluster-level thresholding to control for multiple comparisons using the Monte Carlo simulation tool implemented in Brain Voyager [122]. We then conducted a second GLM analysis in which invisible-target vs visible-target was a repeated measure and AA/day across pregnancy was a continuous predictor. We did not control for sex because performance did not differ between boys and girls.

Because previous studies have found that males and females activate different regions during navigation [226, 249, 250], we identified regions separately in boys and girls where differences in activation between invisible- and visible-target conditions are associated with extent of PAE. Beta maps were created for each subject for the invisible- vs visible-target contrast and analysed separately for boys and girls at the second level in a GLM in relation to AA/day. The voxelwise threshold was set to $p < 0.05$, cluster size corrected. In each identified cluster, mean % signal change was extracted for each subject for each condition to examine in each region whether the association of degree of PAE with the difference in activation between invisible-target and visible-target conditions survives after controlling for potential confounders.

To identify regions crucial for successful place learning, we repeated the second level analysis, again separately for boys and girls, with path length and time to target, respectively, as continuous outcome measures. Mean % signal change values were extracted in the clusters for each subject to examine whether associations survive after controlling for alcohol exposure.

5.2.5. Statistical Analyses

All variables were examined for normality of distribution. The following variables with outliers greater than 3 standard deviations (SD) beyond the mean were transformed by recoding all outlying values to 1 point beyond the highest/lowest observed value: AA/day across pregnancy (n = 1), smoking during pregnancy (n = 1), mother's education (n = 1), postnatal lead exposure (n = 1), mean % signal change differences between invisible and visible conditions in the clusters centered on the right middle frontal gyrus and the bilateral precuneus where boys showed associations with AA/day (n = 1 each), mean % signal change differences between invisible and visible conditions in the clusters centred on the bilateral precuneus and the left middle occipital gyrus where boys showed greater activation than girls (n = 1 each), path length to target (n = 2) and latency (n = 1).

Seven control variables were assessed for consideration as potential confounders of the relation of PAE to spatial navigation: three maternal demographic characteristics (mother's age at delivery and years of education and primary caregiver's marital status), two child characteristics (sex and age at assessment), and two other exposures (maternal smoking during pregnancy and postnatal lead exposure) known to impact on the child's academic performance. Lead exposure, based on a venous blood sample obtained from the child at 5 years, was included because lead levels in this population are within the range in which subtle but meaningful effects on cognitive function have consistently been reported (e.g., [15, 146]. Each control variable that was even weakly related (at $p < 0.10$) to a given outcome measure was considered a potential confounder of the effect of alcohol exposure on the outcome in question.

The outcome measures were latency (in seconds), path length to reach the target (in arena units), and difference in % signal change between the invisible and the visible condition in each of the regions where associations with extent of PAE were found for boys and girls separately. Differences between diagnostic groups (FAS/PFAS; HE; control) were examined for each of the behavioral measures using analyses of variance (ANOVA). *Post hoc* comparisons were computed using the least-squares difference (LSD) approach. Sex differences in each of the behavioural measures were examined using *t*-tests. Sex differences in performance were also examined within each diagnostic group. ANCOVA was used to examine whether effects remained significant when controlling for potential confounders related to the outcome in question at $p < 0.10$. The relation of the continuous measure of PAE across pregnancy, AA/day, to each of the behavioural measures was examined using Pearson correlation analysis, in all the children combined and in boys and girls separately. Multiple regression analyses relating the continuous exposure measure to each of the outcomes, where control variables related to the outcome in question at $p < 0.10$ were controlled for to adjust for potential confounding. All analyses were repeated omitting those children whose mothers used drugs other than alcohol during pregnancy.

5.3. Results

5.3.1. Sample Characteristics

Fifty-seven children were scanned: 27 with FAS or PFAS, 14 HE, and 16 controls. Sample characteristics for these children are summarized in Table 5.1. Whereas mothers of children with FAS/PFAS reported having consumed an average of 7.8 standard drinks of alcohol per drinking occasion during pregnancy as contrasted to 5.2 standard drinks for mothers of the HE group, the FAS/PFAS group consumed alcohol about twice as often as the HE mothers. All but one of the control mothers abstained from drinking during pregnancy; the one light-drinking control consumed 2 drinks on 3 occasions

The groups were generally similar in terms of sample characteristics, with a few exceptions. Mothers of children with FAS/PFAS had less formal education than the other two groups; mothers of the HE group, fewer years of education than mothers of controls. There was little illicit drug use, with three mothers of children with FAS/PFAS reporting use of marijuana during pregnancy and one mother of a child with FAS/PFAS reporting use of cocaine. On average, children with FAS/PFAS had greater lead concentrations than controls. As expected, children with FAS/PFAS scored more poorly on the WISC-IV than HE and control children.

After exclusions due to excessive motion, 41 children provided usable functional data: 19 with FAS/PFAS (9 boys and 10 girls), 10 HE (4 boys and 6 girls), and 12 controls (6 boys and 6 girls). Mean and maximum displacements did not differ between diagnostic groups ($F(2, 38) = 0.245$, $p = 0.784$ and $F(2, 38) = 0.914$, $p = 0.410$, respectively). The children with usable scanner data did not differ from those excluded in terms of alcohol exposure ($t(55) = 1.29$, $p = 0.20$ for AA/day), FASD diagnostic group ($\chi^2(2, N = 57) = 0.11$, $p = 0.95$), performance (all ps > 0.90), or any other demographic variable (all ps > 0.20).

5.3.2. Behavioral Performance

All 57 children in the sample were included in the behavioral analyses, whether or not they provided usable functional imaging data. We only report data from the first trial of the post-scanner navigation test because performance on that trial reflects whether the child learnt the location of the platform during the scan. All children had practised the task extensively before scanning and as such were comfortable with the procedure.

Performance for the boys and girls was similar for the sample as a whole and within each diagnostic group (all ps > 0.20, Table 5.2). No differences in performance were seen between diagnostic groups when looking at all the children ($F(2,54) = 0.58$ for path length and $F(2,54) = 0.70$ for latency, ps > 0.20). Nor were differences by diagnostic group seen in boys ($F(2,26) = 0.64$ for path length and $F(2,26) = 1.40$ for latency , ps > 0.20) or in girls ($F(2,25) = 0.57$ for path length and $F(2,25) = 0.34$ for latency, ps > 0.20), separately.

Table 5.3 shows the relation of the control variables with the behavioural measures. Path length to target and latency were not related (at $p < 0.10$) to any of the control variables except for lead exposure.

The relation of PAE to behavioral performance is shown in Table 5.4, both before and after controlling for lead exposure. There was an overall effect of PAE on latency, largely due to the effect on the boys. In boys, increased alcohol exposure was associated with both longer path length to target and time taken to reach the target. This relation between alcohol exposure and time to target remained significant after controlling for lead exposure. In girls, there was no relation between performance and alcohol exposure levels.

All behavioral findings were essentially unchanged when the analyses were re-run omitting the four children whose mothers used illicit drugs during pregnancy.

5.3.3. Neuroimaging Assessments

Boys showed greater increases than girls in activation during the invisible condition compared to the visible condition in the right middle frontal gyrus and inferior parietal lobule and left precuneus, superior parietal lobule, lingual gyrus, middle occipital gyrus, and middle temporal gyrus (Table 5.5). Between-group differences in activation remained significant after controlling for PAE. There were no regions where girls showed greater differences than boys in activation between the two conditions. Whereas boys showed increased activation in the invisible-target compared to visible-target condition, girls showed reduced activation during the invisible-target condition in diffuse regions across all lobes.

Because we observed differences in brain activation in boys and girls, and because several of our previous imaging studies have shown that continuous measures are often more sensitive than diagnostic categorical measures [37, 82, 158], we examined whether there were regions where differences in activation between the invisible- and visible-target conditions were associated with levels of PAE within each sex separately. In girls, there were no regions where differences in activity were related to degree of alcohol exposure. In contrast, as shown in Table 5.6, in boys increasing alcohol exposure was strongly associated with smaller activation increases between the invisible-target and visible-target conditions in the right parahippocampal gyrus, superior temporal gyrus, transverse temporal gyrus, precentral gyrus, inferior frontal gyrus, middle frontal gyrus, insula and claustrum, bilateral precuneus, caudate, thalamus and lentiform nucleus, and the left posterior cingulate (Figure 5.3, Figure 5.4).

When examining the relation of the control variables with differences in mean % signal change between invisible- and visible-target conditions in each of the regions where associations of activation with level of alcohol exposure were found in boys, only child's age at scan was related at $p < 0.10$ to differences in activation in the cluster centred on the right middle frontal gyrus ($r = 0.27$, $p = 0.08$); all other ps > 0.20 (Table 5.6). After controlling for the potential confounding effects of age in this region, the association with degree of alcohol exposure remained significant.

Table 5.1: Sample characteristics (N = 57).

	FAS/PFAS (*n* = 27)	Heavily exposed (*n* = 14)	Control (*n* = 16)	*F* or χ^2	*p*
Maternal					
Age at delivery[a]	29.0 (7.4)	25.5 (5.0)	25.7 (3.3)	2.37	0.103
Education (years)[b]	7.1 (2.2)	8.6 (3.1)	10.6 (1.6)	11.49	0.001
Marital status (% married)	44.4	35.7	68.8	3.71	0.156
Smoking during pregnancy (cigarettes/day)[c]	8.0 (5.7)	8.4 (7.2)	3.4 (9.9)	2.36	0.104
Prenatal alcohol exposure					
AA/day at conception (oz)[d]	1.8 (2.1)	0.6 (0.6)	0.0 (0.0)	7.18	0.002
AA/occasion at conception (oz)[e]	4.3 (2.5)	2.6 (2.5)	0.1 (0.3)	19.57	0.001
Frequency at conception (days/week)[f]	2.6 (1.7)	1.3 (1.1)	0.0 (0.1)	21.00	0.001
AA/day across pregnancy (oz)[g]	1.2 (1.4)	0.5 (0.5)	0.0 (0.0)	7.66	0.001
AA/occasion across pregnancy (oz)[h]	3.9 (1.9)	2.6 (1.6)	0.1 (0.3)	30.40	0.001
Frequency across pregnancy (days/week)[i]	2.0 (1.4)	1.1 (0.9)	0.0 (0.0)	18.13	0.001
Child					
Sex (% male)	51.9	50.0	50.0	0.02	0.990
Age at scan[j]	9.4 (0.3)	9.6 (0.6)	9.4 (0.4)	2.31	0.109
Blood lead concentration (µg/dl)[k]	11.9 (9.5)	9.5 (3.9)	7.9 (3.0)	3.39	0.041
WISC Estimated Full Scale IQ score[l]	64.5 (9.5)	72.8 (8.2)	76.4 (9.1)	9.43	0.000
Behavioral performance					
Path length to target (arena units within a 500x500 grid)	140.1 (130.1)	148.6 (99.0)	118.3 (132.5)	0.25	0.780
Latency (s)	31.1 (31.7)	27.1 (19.9)	20.8 (20.6)	0.76	0.473

Values are Mean (SD)
FAS = fetal alcohol syndrome; PFAS = partial FAS; AA = absolute alcohol, 1 oz AA ≈ 2 standard drinks.
[a]HE, cont < FAS/PFAS, *p*s < 0.081
[b]FAS/PFAS < cont, $p < 0.0001$; HE < cont, $p=0.027$; FAS/PFAS < HE, $p=0.044$
[c]Cont < FAS/PFAS, HE, *p*s < 0.71
[d]Cont < FAS/PFAS, $p = 0.001$; HE < FAS/PFAS, $p = 0.29$
[e]Cont < FAS/PFAS, $p < 0.0001$; cont < HE, $p = 0.002$; HE < FAS/PFAS, $p = 0.017$
[f]Cont < FAS/PFAS, $p < 0.0001$; HE < FAS/PFAS, $p = 0.002$; cont < HE, $p = 0.011$
[g]Cont < FAS/PFAS, $p < 0.0001$; HE > FAS/PFAS, $p = 0.036$
[h]Cont < FAS/PFAS, HE, both *p*s < 0.0001; HE < FAS/PFAS, $p = 0.016$
[i]Cont < FAS/PFAS, $p < 0.0001$; cont < HE, $p = 0.005$; HE < FAS/PFAS, $p = 0.015$
[j]HE > FAS/PFAS, $p = 0.037$
[k]Cont < FAS/PFAS, $p = 0.014$
[l]FAS/PFAS < cont, $p < 0.0001$; FAS/PFAS < HE, $p = 0.008$

Table 5.2: Comparison of post test performance between boys and girls.

Group	*N*	Boys	Girls	*p*
Path length				
Diagnostic groups combined	29 boys / 28 girls	137.00 (108.27)	122.92 (101.14)	0.614
Comparison within and between Diagnostic groups:				
FAS/PFAS	14 boys / 13 girls	156.6 (127.0)	108.5 (87.7)	0.267
HE	7 boys / 7 girls	138.5 (83.9)	158.7 (118.2)	0.719
Control	8 boys / 8 girls	101.5 (93.2)	115.0 (112.0)	0.797
		ANOVA p = 0.534	ANOVA p = 0.570	
Latency				
Diagnostic groups combined	29 boys / 28 girls	25.98 (26.46)	27.58 (23.42)	0.809
Comparison within and between Diagnostic groups:				
FAS/PFAS	14 boys / 13 girls	34.3 (34.4)	25.6 (22.7)	0.453
HE	7 boys / 7 girls	20.1 (17.3)	34.0 (21.1)	0.202
Control	8 boys / 8 girls	16.6 (8.8)	25.1 (28.2)	0.428
		ANOVA p = 0.265	ANOVA p = 0.717	

Values are Mean (SD)
FAS = fetal alcohol syndrome; PFAS = partial FAS; HE = heavily exposed nonsyndromal.

Table 5.3: Correlation between control variables and behavioural measures for all children.

	N	Child's sex r (p)	Child's age at scan r (p)	Lead exposure r (p)	Maternal age r (p)	Maternal education r (p)	Smoking during pregnancy r (p)	Primary Caregiver's marital status r (p)
All	57							
Path length to target		-0.07 (0.61)	-0.05 (0.72)	0.30 (0.03)	0.08 (0.57)	-0.16 (0.24)	0.02 (0.86)	-0.19 (0.16)
Latency		0.03 (0.81)	-0.15 (0.26)	0.23 (0.09)	0.06 (0.65)	-0.16 (0.23)	-0.02 (0.88)	-0.21 (0.11)

Table 5.4: Relation of degree of prenatal alcohol exposure to behavioural performance.

	AA per day	
	***r* (*p*)**	**ß (*p*)**
All (*N* = 57)		
Path length to target[a]	0.21 (0.113)	0.14 (0.306)
Latency[a]	0.26 (0.051)	0.21 (0.124)
Boys (*N* = 29)		
Path length to target[a]	0.38 (0.044)	0.29 (0.163)
Latency[a]	0.50 (0.006)	0.47 (0.020)
Girls (*N* = 28)		
Path length to target[a]	0.11 (0.584)	0.06 (0.755)
Latency[a]	0.08 (0.699)	0.04 (0.832)

AA = ounces absolute alcohol

[a]controlled for lead exposure

Table 5.5: Regions where the difference in activation between the invisible and visible conditions is greater in boys than in girls (p < 0.01, cluster size corrected, all clusters > 324 voxels). Co-ordinates are Talairach co-ordinates of the peak voxel.

Lobe	Region	BA	x	y	z	No of voxels[a]	Mean *t*
occipital	left lingual gyrus extending to middle occipital gyrus	17, 18	-22	-86	3	394	4.75
parietal	right inferior parietal lobule	40	41	-47	51	354	4.45
parietal	left precuneus extending to superior parietal lobule	7	-19	-71	45	1092	4.75
frontal	right middle frontal gyrus extending to sub-gyral frontal lobe	6	32	-5	48	502	3.97
occipital	left middle occipital gyrus extending to middle temporal gyrus	18, 19, 39	-25	-89	18	1194	4.96

BA=Brodmann area

[a]Voxel size refers to the 1x1x1 mm^3 resolution of the iso-voxeled structural images

first region mentioned = region at the peak voxel, other regions arranged in order of decreasing size

Table 5.6: Regions in boys only where increasing alcohol exposure is associated with smaller activation increases during the invisible condition compared to the visible condition (p < 0.05, cluster size corrected, all clusters > 1026 voxels). Co-ordinates are Tal

Lobe	Region	BA	x	y	z	No of voxels[a]	Mean *r* (*p*)	Mean ß (p)
temporal	right transverse temporal gyrus, extending to superior temporal gyrus and insula	13, 41, 42	47	-26	12	1041	-0.72 (0.001)	-0.72 (0.001)
frontal	right precentral gyrus extending to inferior frontal gyrus, insula and middle frontal gyrus	6, 9, 13, 44	32	-5	29	2182	-0.71 (0.001)	-0.71 (0.001)
basal ganglia	bilateral caudate, extending to thalamus and lentiform nucleus	-	-10	-2	15	2247	-0.71 (0.001)	-0.71 (0.001)
frontal	right middle frontal gyrus extending to claustrum, insula and inferior frontal gyrus [b]	13,46	38	28	18	1082	-0.65 (0.002)	-0.69 (0.001)
limbic	right parahippocampal gyrus extending to lentiform nucleus and claustrum	-	23	-2	-9	1888	-0.71 (0.001)	-0.71 (0.001)
parietal	bilateral precuneus extending to left posterior cingulate	7, 31	8	-53	45	2100	-0.63 (0.004)	-0.63 (0.004)

BA=Brodmann area

[a]Voxel size refers to the 1x1x1 mm^3 resolution of the iso-voxeled structural images

first region mentioned = region at the peak voxel, other regions arranged in order of decreasing size

[b]Controlled for age

Across groups, greater activation increases in boys in the bilateral precuneus and a large left frontal region during the invisible condition compared to the visible condition was associated with improved place learning as reflected both by shorter path lengths (Table 5.7 (a)) and a shorter latency (Table 5.7 (b)). These correlations remained significant after controlling for alcohol exposure (all *p*s < 0.042). In girls, there were no regions where activation increases were associated with better performance.

All imaging findings were essentially unchanged when the analyses were re-run omitting the four children whose mothers used illicit drugs during pregnancy.

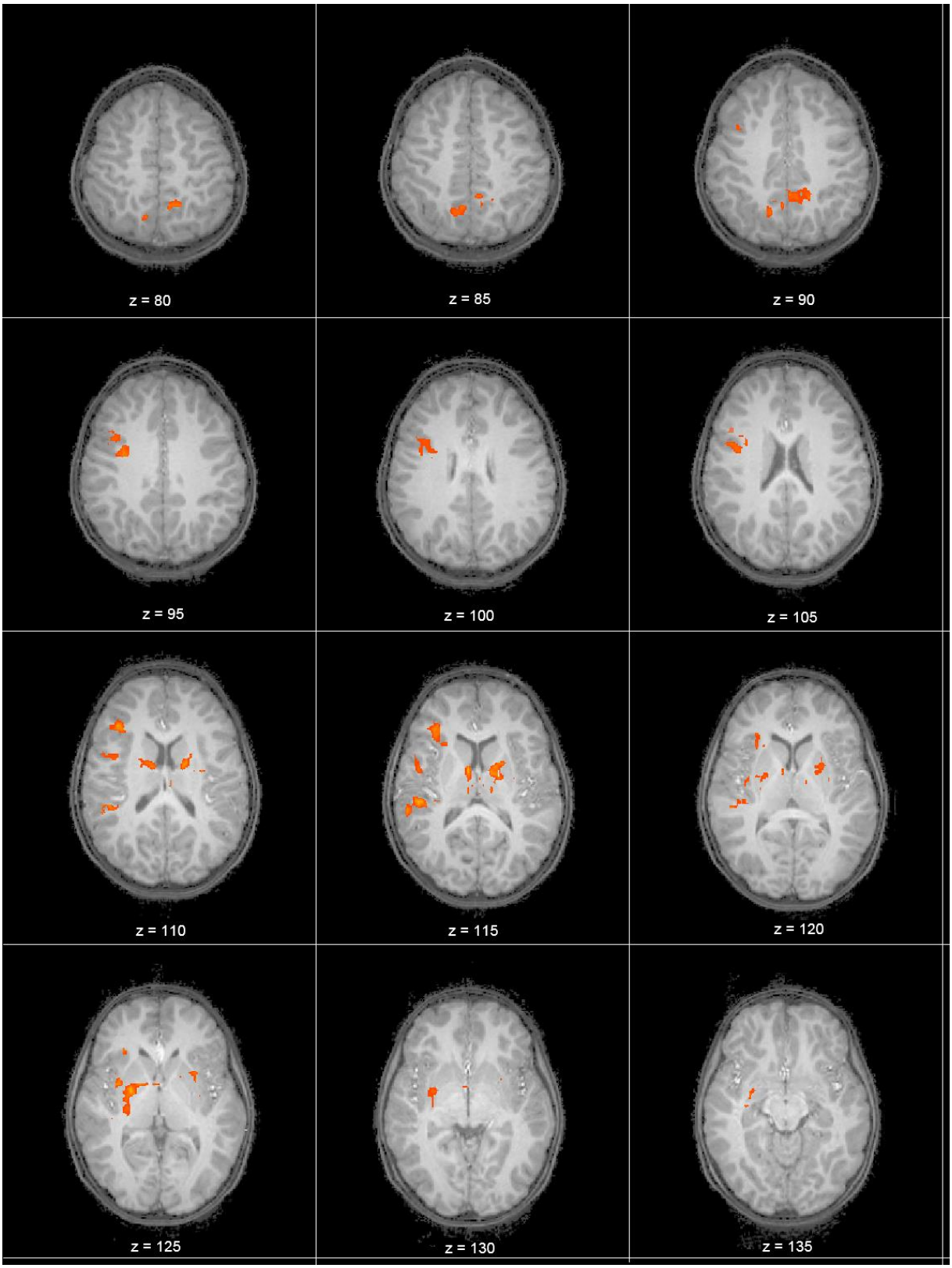

Figure 5.3: Transverse slices showing regions where activation is negatively related to absolute alcohol per day in boys only (z = Talairach co-ordinate).

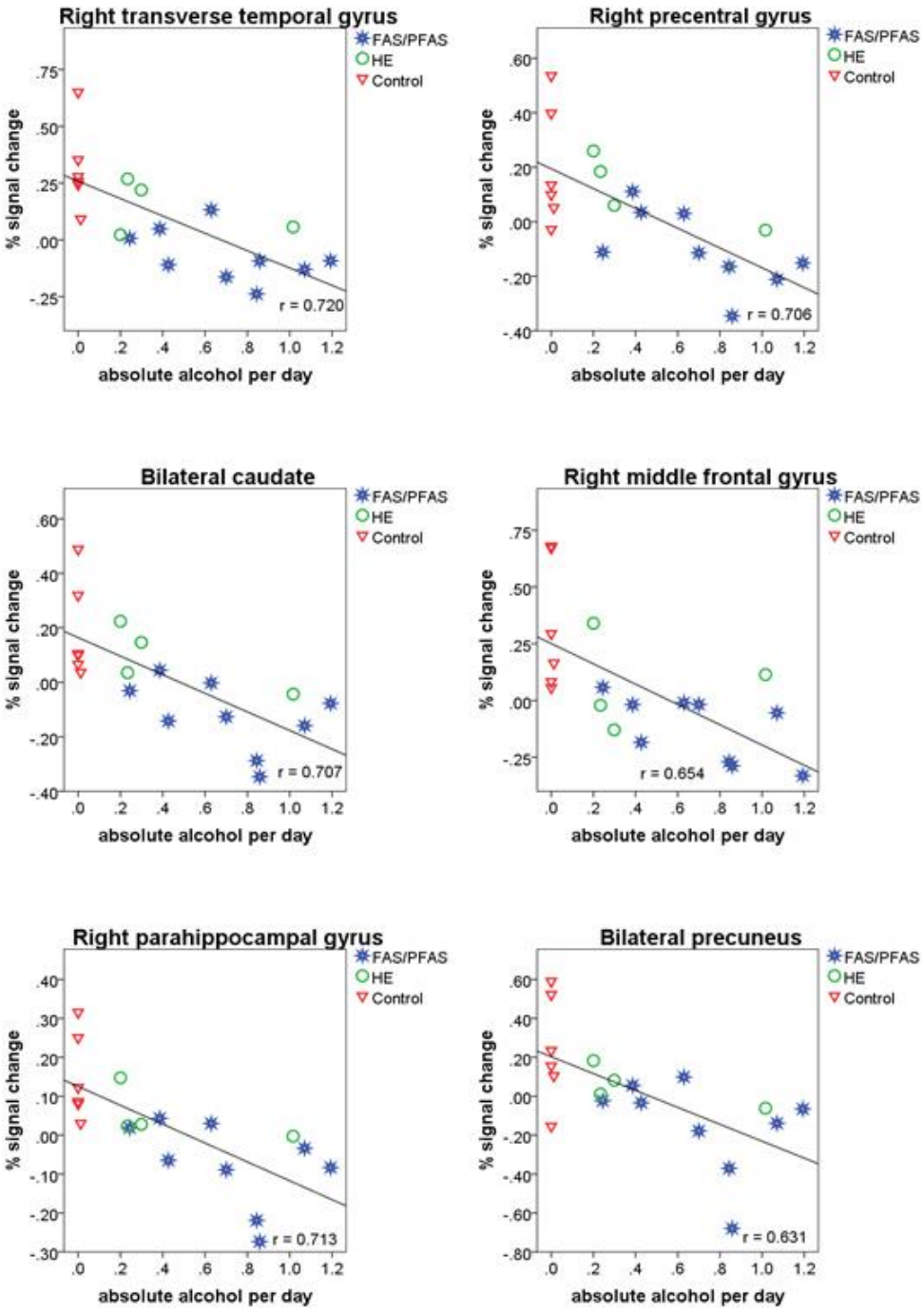

Figure 5.4: Relation of absolute alcohol per day to percent signal change in boys only.

Table 5.7: Regions in boys only *where smaller activation increases during the invisible condition compared to the visible condition are associated with (a) longer path lengths and (b) increasing latency (*p *< 0.05, cluster size corrected). Co-ordinates are Talairach co-ordinates of the peak voxel.*

a)

Lobe	Regions associated with path length	BA	x	y	z	No of voxels[a]	Mean *r* (*p*)	Mean ß (*p*)[b]
parietal	bilateral precuneus extending to left cuneus	7, 31	-4	-65	39	1869	-0.61 (0.005)	-0.48 (0.035)
frontal	left superior frontal gyrus extending to medial frontal gyrus, middle frontal gyrus, cingulate gyrus	6, 8, 9, 10, 32	-16	43	33	2312	-0.59 (0.008)	-0.45 (0.042)

b)

Lobe	Regions associated with latency	BA	x	y	z	No of voxels[a]	Mean *r* (*p*)	Mean ß (p)[b]
frontal	left anterior cingulate extending to medial frontal gyrus, middle frontal gyrus, superior frontal gyrus	6, 8, 9, 10, 24, 32	-4	19	24	3370	-0.67 (0.002)	-0.64 (0.006)
parietal	bilateral precuneus extending to cuneus	7	8	-56	51	1520	-0.59 (0.008)	-0.55 (0.022)

BA=Brodmann area
[a]Voxel size refers to the 1x1x1 mm^3 resolution of the iso-voxeled structural images
first region mentioned = region at the peak voxel, other regions arranged in order of decreasing size
[b]Controlled for absolute alcohol per day across pregnancy

5.4. Discussion

Although we detected no sex differences in behavioral performance, activation increases during the invisible condition compared to the visible condition were greater in boys than in girls in parietal regions, including the precuneus and the superior and inferior parietal lobules, as well as in frontal and occipital regions. In contrast, no regions showed greater activation increases during the invisible condition in girls. In boys, PAE was associated with poorer place learning and reduced activation increases during the invisible condition in the parahippocampal gyrus, precuneus, posterior cingulate, frontal and temporal lobes, caudate, insula, claustrum, lentiform nucleus and thalamus, with most of these being right lateralised. Notably, better place learning in boys was associated with greater activation increases during the invisible condition in a precuneus region similar to that showing PAE effects, as well as a large left frontal region. In girls, PAE was not associated with performance or activation differences between invisible and visible conditions in any regions; nor was better performance related to activation differences between conditions in any regions.

Although most studies have found better allocentric navigational performance in males, including pre-pubertal children [248], in computer-simulated versions of the Morris water maze [105, 240-247, 256], a few have found no sex differences in performance [106, 107, 250]. It is possible that inclusion of a probe trial, where the platform is removed unbeknownst to the participants, may have increased sensitivity in detecting sex differences in these studies as a male advantage has been demonstrated previously in probe trials even in the absence of performance differences in invisible-target trials [241, 242].

Despite the absence of sex differences in performance, our finding that there was no greater increased activation during invisible conditions in girls than boys in any regions, compared to greater increases in activity during invisible conditions

in parietal, frontal and occipital regions in boys, provide further evidence that males and females use different navigation strategies [257-259] and activate different brain regions [250] during place learning. Females tend to navigate egocentrically, using mainly landmark cues, whereas males tend to navigate allocentrically, using mainly Euclidean information, such as direction, distance, gradient (slope of the floor), and geometry [105, 226, 246, 259-264].

Although effects of PAE on place learning have been reported in studies involving both males and females [85], at least three other studies have reported effects in males only – one, an animal study, in which only male rats showed poorer performance in a T-maze [253]; another in which only male rats were examined and which showed poorer and perseverative performance on the MWM by those moderately exposed to alcohol [265]; and the third, a human study which also did not include girls and which found that boys with FAS took longer paths to find a hidden platform [61].

PAE has been shown to have a greater impact on spatial location memory than on object memory. For example, Uecker and Nadal [92, 93] showed impairment in memory for object location in children with FASD, though not in the recall of the objects themselves, a finding replicated by Kaemingk and Tanner Halverson [94]. Poorer performance has also been demonstrated on the Visual Learning task from the Wide Range Assessment of Memory and Learning [99], in which subjects are asked to remember the location of designs [4, 95]. The findings that PAE appears to affect location memory specifically and that males rely more on spatial strategies for navigation may explain why their performance is impaired by PAE, whereas performance of females, who rely more on landmarks (object memory), is largely unaffected.

Notably, the right parahippocampal gyrus, where PAE was related to reduced activation, has been shown to mediate allocentric navigation [217, 219, 220, 238, 264, 266-268], which tends to be employed more by males than females [259, 260, 264]. A number of other regions showing PAE effects in boys, including the precuneus [269], posterior cingulate [220, 270], caudate [271], precentral gyrus [272] and thalamus [268], are also associated with allocentric spatial memory.

Place learning in boys may be more affected by PAE due to the fact that they rely to a greater extent on brain regions more vulnerable to alcohol. Notably, two of the regions affected by PAE in boys, the left precuneus and the right middle frontal gyrus (see Table 5.6), are amongst the regions showing greater activation increases during the invisible condition compared to the visible condition in boys than girls (Table 5.5). Increased activation during invisible conditions in a similar region in the precuneus was also associated with better place learning performance in boys only (Table 5.7), suggesting that in boys the use of the precuneus is an efficient strategy, while it is not in girls. To explore this idea further, we compared brain activation in unexposed boys and girls in those regions showing alcohol related impairment in boys. All the regions showing alcohol-related alterations in activation in boys were activated more by unexposed boys than unexposed girls. These results suggest that because unexposed boys, more than unexposed girls, rely on regions vulnerable to the effects of PAE, due to different strategies employed, their performance is more impaired. It is likely that the regions preferentially activated by control girls are less affected by prenatal alcohol exposure.

A limitation of this study was the relatively small sample sizes of the groups, especially the HE and control groups. Sample sizes were smaller than planned because about 28% of the children (*n* = 16) were excluded from the functional analysis due to motion. Another limitation is the fact that we scanned the children only while they were passively learning the arena and not while actively navigating. We followed this procedure because it is very difficult to have the children navigate with a joystick and remain still at the same time – even during the passive task we had problems with excessive motion. Furthermore, our post-test navigation task did not include a probe trial to test learning. Performance on that trial may have been more sensitive to sex differences in performance and could have been used to examine differences in navigation strategies.

Future studies could include an egocentric version of the arena, as well as the allocentric version used here. It would be interesting to see whether PAE affects the performance and neural correlates of boys and/or girls in the egocentric version.

5.5. Conclusions

Girls and boys are known to use different navigation strategies. These data show that they are activating different brain regions and suggest that the regions used by boys are more susceptible to prenatal alcohol exposure damage, while the regions used by girls are relatively spared. As such, place learning may only be affected by alcohol exposure during pregnancy in boys.

Chapter 6 : Discussion and conclusion

Parietal lobe function

Both number processing and visuospatial skills rely on intact parietal lobe functioning.

The parietal lobe is involved in many aspects of visuospatial processing, including spatial attention [54, 273-275], spatial memory [276-278], mental imagery [279, 280], mental rotation [281-283], saccadic eye movements [170, 284], reaching and grasping [170], pointing [170], visual search [285, 286], and visuospatial transformations [287, 288]. Of particular interest here is the parietal lobe's involvement in spatial navigation and spatial location memory, which both play a role during tasks such as virtual versions of the Morris water maze [238, 239, 272]. The posterior parietal cortex is involved in an egocentric representation of the environment [225], with the precuneus also being involved in allocentric spatial processing [269]. The posterior cingulate is involved in the transformation from an egocentric frame of reference in the parietal lobe to an allocentric frame of reference [289-292].

As discussed previously, Dehaene *et al.* [50] identified 5 main regions of the parietal lobe activated during number processing: the left and right IPS, the left angular gyrus, and the left and right PSPL. A 2011 meta-analysis [293], however, showed that additional parietal regions play a role during number processing, including the bilateral precuneus, inferior parietal lobe, postcentral gyrus and right supramarginal gyrus.

Link between number and spatial processing

Considering that both number and spatial processing rely on the parietal lobe, it is not surprising that there are behavioural links between them.

Numbers are intuitively visualized from left to right on a spatial number line in people who read from left to right [294]. This mental number line is evidenced by the spatial-numerical association of response codes (SNARC) effect: relatively large numbers are responded to more quickly with the right hand, while relatively small numbers are responded to more quickly with the left hand [132, 295]. The SNARC effect is observed in a variety of tasks, whether or not numerical magnitude is relevant to the task, including parity judgement (odd or even) [295, 296], magnitude comparison [297], phoneme detection using number words [296, 298], and judging whether numbers are the same or different [299]. The effect is apparent even when a digit unrelated to the task is shown in the background [298, 300], which suggests automatic access to the mental number line representation [301]. The SNARC effect does not just hold for Arabic numbers, but has been demonstrated when using different notations: number words [295, 298], nonsymbolic number [302], and even with auditory presentation [302]. The effect is present in both left and right handers [295] and is not affected when the hands are crossed [295]. However, the SNARC effect is affected by reading direction: it is reversed in Iranian subjects who read from right to left [295]. The SNARC effect provides evidence for spatial-congruency between the response side in egocentric space and the position of the numerical value on a mental number line [301].

Viewing numbers can bias visual attention in a direction congruent with that number's position on the number line, i.e. small numbers bias attention to the left side, while large numbers bias attention to the right side. Targets on the left side are detected more quickly after viewing a small number, while targets on the right side are detected more quickly after viewing a large number [303, 304]. In a temporal order judgment (TOJ) task, participants indicated whether a left or right stimulus appeared first. When the stimuli appeared simultaneously, more left sided responses were recorded after actively attending to a small number [305]. When judging which side of a transected line is longer, viewing small numbers caused more correct responses when the left side was longer, while viewing larger numbers caused more incorrect responses when the left side was longer [306].

Studies of people with left hemifield neglect, resulting from right parietal lesions, provide further evidence for the relation between numbers and space. Patients with left neglect tend to ignore the left portion of space, for example when they bisect physical lines they neglect the left portion of the line and tend to place the midpoint too far to the right [227, 307]. These patients show similar neglect of the left portion of the number line when performing number bisection, overestimating the midpoint, even though the numbers are given to them verbally [308]. When performing number comparison, patients with left neglect are slower to decide whether the number immediately to the left of the target (i.e. smaller than the target) is greater or less than the target than when the number is immediately to the right [309].

Comparison of brain activations during visuospatial and number processing

Despite the above evidence of a relation between numbers and space, the regions found to be affected by prenatal alcohol exposure in the mathematical tasks in our studies did not overlap with any of the regions affected by prenatal alcohol exposure in the CG arena task. The bilateral precuneus was the only parietal region where effects of prenatal alcohol exposure were found during administration of the CG arena task, with higher levels of alcohol exposure being related to smaller activation increases during the invisible condition in boys. Since in the number processing tasks we only examined effects of prenatal alcohol exposure on activation in *a priori* defined ROIs that did not include the precuneus, compared to a whole-brain analysis in the CG arena task, it is not surprising that parietal regions showing alcohol effects in the number processing and CG arena tasks did not overlap. We do not know whether activation of the precuneus during number processing was affected by prenatal alcohol exposure as it was not directly investigated. Since the precuneus is known to be involved in number processing tasks [293], as well as encoding and retrieval of spatial location [269], it may be involved in a similar way in number line position and spatial location. Notably, the PSPL discussed by Dehaene [50] extends into the precuneus and the right PSPL ROI used in the ROI analysis in our math studies is in close proximity to the precuneus region showing alcohol effects in the CG Arena task, suggesting that a similar region of the precuneus might in fact be affected by prenatal alcohol exposure during number processing. In a previous whole-brain analysis of a subset of the children studied here, children with FAS/PFAS showed greater activation

increases in the precuneus during proximity judgment than controls, which was attributed to greater effort required by these children to complete this relatively simple math task due to damage in regions specialized for number processing.

To explore whether the region showing alcohol effects in boys in the CG Arena task may also show alcohol effects during number processing, we extracted for each of the math tasks beta values in that bilateral precuneus region. Activation increases during symbolic number processing in this region did not show differences between exposed and unexposed children or associations with alcohol exposure levels (all p's > 0.7). The absence of alcohol effects may be due to the fact that this region is much larger (2100 mm^3) than the regions showing alcohol effects in the Meintjes et al. [60] paper (two regions, sizes 531 and 119 mm^3, respectively). Further only the right precuneus from that paper overlaps directly with this region, and they only found alcohol effects in the precuneus when controlling for IQ.

In contrast, in the Smarties nonsymbolic number comparison (NC) task, greater prenatal alcohol exposure was associated with smaller activation increases during NC in this region (r = -0.54, p = 0.003 for AA/day; and r = -0.48, p = 0.011 for AA/occasion). Notably, *all* children showed alcohol-related reductions in activation in this region during NC, not just the boys as was the case in the CG arena task. These results support our conclusion that the absence of alcohol effects in the precuneus in girls during the CG arena task is a consequence of the fact that they use different navigation strategies that do not rely on activation of this region.

Since the precuneus is involved in a variety of visuospatial tasks, including spatial attention, visual rotation, mental imagery, and mental navigation [310], it is possible that only NC showed an alcohol effect being more visual than the other math tasks. Supporting this, in a meta-analysis of number processing in children, the left precuneus was shown to be activated during nonsymbolic number processing, but not during symbolic number processing [135].

With the increased power of ROI analyses, it is possible that some of the number processing ROIs may have shown alcohol effects during the CG arena task. To explore this, for each of the *a priori* defined number processing ROIs we extracted the beta values for the invisible vs visible condition. Of the regions showing activation increases during the invisible condition, none revealed group differences or associations with alcohol exposure (all p's > 0.14). This was true whether considering all the children, or boys and girls separately.

Comparison of brain activations during symbolic and nonsymbolic number processing

While we found that the activation of the right IPS was impaired by PAE during both symbolic and nonsymbolic number processing, we found no relationship between alcohol or diagnostic group and activation of the left IPS. A question that arises is whether the right IPS is more affected by prenatal alcohol exposure than the left IPS, or whether they are equally affected but only the effect on the right IPS is apparent during number processing because the left IPS is recruited to a lesser extent by these tasks.

In the Dehaene meta-analysis [50] used to identify the critical parietal number processing regions used in our studies, the IPS activation was bilateral in all the studies except two. One of these found increases on the left only when adding large numbers compared to small numbers [155], and the other increases on the right during numerosity estimation compared to physical matching [311].

Although the previous two studies of number processing in FASD reported regions impaired by prenatal alcohol exposure in close proximity to our right IPS ROI [60, 81], neither found an alcohol effect on the left. Meintjes *et al.* [60] found that although both the right and left IPS were activated during PJ, only the right IPS showed reduced activation by children with FAS/PFAS compared to controls. Similarly, while both the left and right inferior parietal lobules (in close proximity to the IPS) were activated during subtraction, only the right showed greater activation by controls than those with alcohol related dysmorphology [81]. Together with our own findings here of PAE effects only in the right IPS during both symbolic and nonsymbolic number processing, these results suggest that, despite bilateral activation of the IPS during number processing, only the right IPS is affected by prenatal alcohol exposure while the left IPS is relatively spared.

To examine whether this absence of an alcohol effect on the left may be due to differences in the degree of activation on the left and right, we compared the magnitudes of the activation in the left and right IPS in control children during symbolic and nonsymbolic number processing. In control children, neither the left nor the right IPS showed significant activation increases during nonsymbolic number processing. One-sample Student *t*-tests on the percent signal change during PJ relative to the control condition in the right and left IPS ROIs showed that the activation increases in control children in both the right and left IPS ROIs, albeit small, were significant (right IPS mean % signal change ± SD = 0.09 ± 0.12, $p = 0.007$; and left IPS mean % signal change ± SD = 0.07 ± 0.13, $p = 0.045$). In contrast, during EA, activation of the right IPS was stronger and significant (right IPS mean % signal change ± SD = 0.07 ± 0.08, $p = 0.003$), while that on the left was not (left IPS mean % signal change ± SD = 0.02 ± 0.13, $p = 0.519$). Reduced differential activation of the left IPS region may therefore explain the failure to detect PAE effects in this region and more work is required.

While during PJ and Smarties, exposed children showed compensatory increased activation of the left AG, they did not show any compensatory activation during EA. These results are similar to a previous study [60] that also found increased activation of the left angular gyrus in children with FAS/PFAS compared to control children during PJ, but not during EA.

Putting these results together, it seems as if exposed children show compensatory activation of the left angular gyrus during magnitude comparison tasks, both symbolic and nonsymbolic, but not during arithmetic tasks relying on verbal recall. While for magnitude comparison tasks, the control children would use the optimal strategy, relying on the core quantity system in the IPS, the alcohol exposed children appear to recruit the left angular gyrus to a greater extent due to PAE damage to the IPS. In contrast, for arithmetic tasks, verbal recall of number facts (relying on the left angular

gyrus) is the most efficient strategy and appears to be used by all children. These results suggest that the left angular gyrus is not affected by PAE.

While prenatal alcohol exposure was associated with lower activation of the right PSPL during the smarties task, no association with alcohol exposure was found in this region during either the PJ or EA tasks, and this region has not previously been shown to be affected by prenatal alcohol exposure [60, 81]. In contrast, we found alcohol related reductions in activation in the left PSPL during EA [82], and in a comparable region during subtraction [81].

While it appears that both the left and right PSPL are impaired by prenatal alcohol exposure, the impairment of the right PSPL was only evident during nonsymbolic quantity comparison, while impairment of the left PSPL has been observed during symbolic arithmetic operations. Neither of the studies using the PJ task to assess symbolic magnitude comparison found alcohol related impairment in the left nor the right PSPL [60, 82]. Another symbolic number comparison task (for example, a symbolic version of the smarties task) could provide further insight into the lateralization of PAE effects on the PSPL. If prenatal alcohol related impairment was seen in the left PSPL, it would suggest differential impairment for symbolic and nonsymbolic domains: left impairment for symbolic number processing and right impairment for nonsymbolic number processing. Conversely, impairment of the right PSPL would suggest that PAE affects PSPL activation differently for arithmetic and number comparison: left impairment during arithmetic operations and right impairment during number comparison.

The Dehaene meta-analysis does not provide much insight into the laterality of PSPL activation [50], with 3 studies showing bilateral PSPL activation [49, 151, 182], and one study (subliminal quantity priming across notations [312]) showing only right PSPL activation.

Limitations and future work

Only number processing and spatial navigation were examined in this study. In addition to visuospatial processing and number processing, the parietal lobe is also involved in many other domains, including language processing [51], response inhibition [52, 53], non-spatial working memory [54], episodic memory [55], alertness [56], task switching [57], attention [58] and time perception [59]. Using a wider range of tasks would provide more information on the effect of prenatal alcohol on more functional domains and provide a more comprehensive picture of alcohol related parietal dysfunction.

A major limitation of this study relates to the fact that the number processing studies used ROI analyses, while a whole-brain analysis was used for the spatial navigation study. This limits our ability to compare results from these studies directly. Since most studies of brain activation during spatial navigation have focused on the hippocampus, it is not known which parietal regions are involved, making the selection of appropriate ROIs difficult. Future work should

include a whole brain analysis of the number processing data to provide more insight into the effects of prenatal alcohol exposure on other parts of the parietal lobe, as well as other regions of the brain.

Prenatal alcohol exposure is associated with decreased brain volume [30], as well as brain shape abnormalities [45], which could complicate the interpretation of the imaging data. Lower brain volumes in children with FASD could cause the non-affine transformations during the normalization procedure to be non-linear, which could potentially yield outcome differences related to differences in this transformation.

Another limitation of this study was that, by contrast to most of the studies our group has conducted in Cape Town, the maternal report of drinking during pregnancy was obtained retrospectively several years after the child's birth for the children in the math studies. Nevertheless, the validity of these reports is supported by the fact that they have been shown to be predictive of alcohol-related neuroimaging, neurobehavioral, and ERP outcomes in numerous other studies (see for example, [60, 140, 313-316]).

Here we only looked at one task involving spatial location memory. To the author's knowledge, the only other task involving spatial location memory that has been investigated in FASD is the spatial *n*-back task [76-80]. It would be informative to look at the effect of prenatal alcohol exposure on the neural correlates of mental rotation or an egocentric version of the CG Arena task.

Our findings suggest that during math tasks children prenatally exposed to alcohol compensate for the impairment of the right IPS by using verbal strategies that engage the left angular gyrus. This hypothesis could be tested by including a verbal distractor task in future experiments. This would involve interleaving the math tasks with a task that taxes verbal resources, such as a task requiring syntactic judgments of words [317]. It is possible that during arithmetic the verbal distractor task would impair the performance of control and exposed children, while during a magnitude comparison task the performance of only children with prenatal alcohol exposure would be impaired.

Conclusion

In conclusion, we found prenatal alcohol exposure based impairments in the parietal lobe in all of the tasks investigated, with the impairments being widespread throughout the parietal lobe bilaterally. Of the regions investigated in the number processing tasks, only two showed no impairment. The left AG appears to be unimpaired and may serve to compensate for impairments in other parietal regions. Although no evidence of left IPS impairment was found, this may be due to reduced recruitment of this region when performing the task, rather than the absence of an alcohol effect.

Though only one parietal region (the precuneus) was shown to be impaired during the spatial navigation task, this was a large bilateral region. Notably, the bilateral precuneus was affected by prenatal alcohol exposure in both the spatial navigation and smarties tasks. It is possible that this is a key region linking the deficits in number processing and visuospatial skills in children with prenatal alcohol exposure.

The parietal lobe has been shown to be particularly affected by prenatal alcohol exposure, both in macrostructure [30, 45] and in white matter microstructure [89]. This work shows that functionally the effects of prenatal alcohol exposure is widespread throughout the parietal lobe, and have shed some light on particular regions affected. Our findings highlight the vulnerability of the parietal lobe to the effects of prenatal alcohol exposure.

References

[1] A. P. Streissguth, H. M. Barr, and P. D. Sampson, "Moderate prenatal alcohol exposure: Effects on child IQ and learning problems at age 7 1/2 years," *Alcoholism: Clinical and Experimental Research,* vol. 14, no. 5, pp. 662-669, 1990.

[2] S. W. Jacobson, J. L. Jacobson, R. J. Sokol, L. M. Chiodo, and R. Corobana, "Maternal age, alcohol abuse history, and quality of parenting as moderators of the effects of prenatal alcohol exposure on 7.5-year intellectual function," *Alcoholism: Clinical and Experimental Research,* vol. 28, no. 11, pp. 1732-1745, 2004.

[3] S. N. Mattson, E. P. Riley, L. Gramling, D. C. Delis, and K. L. Jones, "Neuropsychological comparison of alcohol-exposed children with or without physical features of fetal alcohol syndrome," *Neuropsychology,* vol. 12, no. 1, p. 146, 1998.

[4] S. N. Mattson and T. M. Roebuck, "Acquisition and retention of verbal and nonverbal information in children with heavy prenatal alcohol exposure," *Alcoholism: Clinical and Experimental Research,* vol. 26, no. 6, pp. 875-882, 2002.

[5] S. N. Mattson, L. Gramling, E. P. Riley, D. C. Delis, and K. L. Jones, "Global—local processing in children prenatally exposed to alcohol," *Child Neuropsychology,* vol. 2, no. 3, pp. 165-175, 1996.

[6] P. Kodituwakku, N. Handmaker, S. Cutler, E. Weathersby, and S. Handmaker, "Specific impairments in self-regulation in children exposed to alcohol prenatally," *Alcoholism: Clinical and Experimental Research,* vol. 19, no. 6, pp. 1558-1564, 1995.

[7] S. N. Mattson, A. M. Goodman, C. Caine, D. C. Delis, and E. P. Riley, "Executive functioning in children with heavy prenatal alcohol exposure," *Alcoholism: Clinical and Experimental Research,* vol. 23, no. 11, pp. 1808-1815, 1999.

[8] T. M. Roebuck, R. W. Simmons, S. N. Mattson, and E. P. Riley, "Prenatal exposure to alcohol affects the ability to maintain postural balance," *Alcoholism: Clinical and Experimental Research,* vol. 22, no. 1, pp. 252-258, 1998.

[9] C. D. Coles, K. A. Platzman, M. E. Lynch, and D. Freides, "Auditory and visual sustained attention in adolescents prenatally exposed to alcohol," *Alcoholism: Clinical and Experimental Research,* vol. 26, no. 2, pp. 263-271, 2002.

[10] S. W. Jacobson, J. L. Jacobson, and R. J. Sokol, "Effects of fetal alcohol exposure on infant reaction time," *Alcoholism: Clinical and Experimental Research,* vol. 18, no. 5, pp. 1125-1132, 1994.

[11] S. W. Jacobson, J. L. Jacobson, R. J. Sokol, S. S. Martier, and J. W. Ager, "Prenatal alcohol exposure and infant information processing ability," *Child Development,* vol. 64, no. 6, pp. 1706-1721, 1993.

[12] A. P. Streissguth, H. M. Barr, H. C. Olson, P. D. Sampson, F. L. Bookstein, and D. M. Burgess, "Drinking during pregnancy decreases word attack and arithmetic scores on standardized tests: Adolescent data from a population-based prospective study," *Alcoholism: Clinical and Experimental Research,* vol. 18, no. 2, pp. 248-254, 1994.

[13] C. D. Coles, R. T. Brown, I. E. Smith, K. A. Platzman, S. Erickson, and A. Falek, "Effects of prenatal alcohol exposure at school age. I. Physical and cognitive development," *Neurotoxicology and Teratology,* vol. 13, no. 4, pp. 357-367, 1991.

[14] M. J. Burden, S. W. Jacobson, and J. L. Jacobson, "Relation of prenatal alcohol exposure to cognitive processing speed and efficiency in childhood," *Alcoholism: Clinical and Experimental Research,* vol. 29, no. 8, pp. 1473-1483, 2005.

[15] L. M. Chiodo, S. W. Jacobson, and J. L. Jacobson, "Neurodevelopmental effects of postnatal lead exposure at very low levels," *Neurotoxicology and Teratology,* vol. 26, no. 3, pp. 359-371, 2004.

[16] J. L. Jacobson, N. C. Dodge, M. J. Burden, R. Klorman, and S. W. Jacobson, "Number processing in adolescents with prenatal alcohol exposure and ADHD: differences in the neurobehavioral phenotype," *Alcoholism: Clinical and Experimental Research,* vol. 35, no. 3, pp. 431-442, 2011.

[17] H. E. Hoyme *et al.*, "A practical clinical approach to diagnosis of fetal alcohol spectrum disorders: clarification of the 1996 institute of medicine criteria," *Pediatrics,* vol. 115, no. 1, pp. 39-47, 2005.

[18] K. R. Stratton, C. J. Howe, and F. C. Battaglia, *Fetal alcohol syndrome: Diagnosis, epidemiology, prevention, and treatment*. National Academies, 1996.

[19] P. A. May *et al.*, "The epidemiology of fetal alcohol syndrome and partial FAS in a South African community," *Drug and Alcohol Dependence,* vol. 88, no. 2, pp. 259-271, 2007.
[20] P. Lemoine, H. Harousseau, J. Borteyru, and J. Menuet, "Les enfants de parents alcooliques: Anomalies observees a propos de 127 cas," *Ouest Méd,* vol. 21, no. 476-482, p. 2, 1968.
[21] K. Jones, D. Smith, C. Ulleland, and A. Streissguth, "Pattern of malformation in offspring of chronic alcoholic mothers," *The Lancet,* vol. 301, no. 7815, pp. 1267-1271, 1973.
[22] C. Guerri, A. Bazinet, and E. P. Riley, "Foetal alcohol spectrum disorders and alterations in brain and behaviour," *Alcohol and Alcoholism,* p. agn105, 2009.
[23] X. Chen, C. D. Coles, M. E. Lynch, and X. Hu, "Understanding specific effects of prenatal alcohol exposure on brain structure in young adults," *Human Brain Mapping,* vol. 33, no. 7, pp. 1663-1676, 2012.
[24] M. Rajaprakash, M. M. Chakravarty, J. P. Lerch, and J. Rovet, "Cortical morphology in children with alcohol-related neurodevelopmental disorder," *Brain and Behavior,* vol. 4, no. 1, pp. 41-50, 2014.
[25] F. F. Roussotte *et al.*, "Regional brain volume reductions relate to facial dysmorphology and neurocognitive function in fetal alcohol spectrum disorders," *Human Brain Mapping,* vol. 33, no. 4, pp. 920-937, 2012.
[26] C. D. Coles *et al.*, "Memory and brain volume in adults prenatally exposed to alcohol," *Brain and Cognition,* vol. 75, no. 1, pp. 67-77, 2011.
[27] A. Nardelli, C. Lebel, C. Rasmussen, G. Andrew, and C. Beaulieu, "Extensive deep gray matter volume reductions in children and adolescents with fetal alcohol spectrum disorders," *Alcoholism: Clinical and Experimental Research,* vol. 35, no. 8, pp. 1404-1417, 2011.
[28] S. L. Fryer, S. N. Mattson, T. L. Jernigan, S. L. Archibald, K. L. Jones, and E. P. Riley, "Caudate volume predicts neurocognitive performance in youth with heavy prenatal alcohol exposure," *Alcoholism: Clinical and Experimental Research,* vol. 36, no. 11, pp. 1932-1941, 2012.
[29] C. Lebel *et al.*, "A longitudinal study of the long-term consequences of drinking during pregnancy: heavy in utero alcohol exposure disrupts the normal processes of brain development," *The Journal of Neuroscience,* vol. 32, no. 44, pp. 15243-15251, 2012.
[30] S. L. Archibald, C. Fennema-Notestine, A. Gamst, E. P. Riley, S. N. Mattson, and T. L. Jernigan, "Brain dysmorphology in individuals with severe prenatal alcohol exposure," *Developmental Medicine & Child Neurology,* vol. 43, no. 03, pp. 148-154, 2001.
[31] V. W. Swayze *et al.*, "Magnetic resonance imaging of brain anomalies in fetal alcohol syndrome," *Pediatrics,* vol. 99, no. 2, pp. 232-240, 1997.
[32] P. De Zeeuw, F. Zwart, R. Schrama, H. Van Engeland, and S. Durston, "Prenatal exposure to cigarette smoke or alcohol and cerebellum volume in attention-deficit/hyperactivity disorder and typical development," *Translational Psychiatry,* vol. 2, no. 3, p. e84, 2012.
[33] V. A. Cardenas *et al.*, "Automated cerebellar segmentation: Validation and application to detect smaller volumes in children prenatally exposed to alcohol," *NeuroImage: Clinical,* vol. 4, pp. 295-301, 2014.
[34] E. P. Riley, S. N. Mattson, E. R. Sowell, T. L. Jernigan, D. F. Sobel, and K. L. Jones, "Abnormalities of the corpus callosum in children prenatally exposed to alcohol," *Alcoholism: Clinical and Experimental Research,* vol. 19, no. 5, pp. 1198-1202, 1995.
[35] E. R. Sowell, T. L. Jernigan, S. N. Mattson, E. P. Riley, D. F. Sobel, and K. L. Jones, "Abnormal development of the cerebellar vermis in children prenatally exposed to alcohol: size reduction in lobules I–V," *Alcoholism: Clinical and Experimental Research,* vol. 20, no. 1, pp. 31-34, 1996.
[36] E. R. Sowell, S. Mattson, P. Thompson, T. Jernigan, E. Riley, and A. Toga, "Mapping callosal morphology and cognitive correlates Effects of heavy prenatal alcohol exposure," *Neurology,* vol. 57, no. 2, pp. 235-244, 2001.
[37] E. Meintjes *et al.*, "A tensor-based morphometry analysis of regional differences in brain volume in relation to prenatal alcohol exposure," *NeuroImage: Clinical,* vol. 5, pp. 152-160, 2014.
[38] E. R. Sowell *et al.*, "Voxel-based morphometric analyses of the brain in children and adolescents prenatally exposed to alcohol," *Neuroreport,* vol. 12, no. 3, pp. 515-523, 2001.
[39] Y. Yang *et al.*, "Abnormal cortical thickness alterations in fetal alcohol spectrum disorders and their relationships with facial dysmorphology," *Cerebral Cortex,* vol. 22, no. 5, pp. 1170-1179, 2012.
[40] D. Zhou *et al.*, "Developmental cortical thinning in fetal alcohol spectrum disorders," *Neuroimage,* vol. 58, no. 1, pp. 16-25, 2011.

[41] A. Fernández-Jaén *et al.*, "Cortical thickness in fetal alcohol syndrome and attention deficit disorder," *Pediatric Neurology,* vol. 45, no. 6, pp. 387-391, 2011.

[42] E. R. Sowell, S. N. Mattson, E. Kan, P. M. Thompson, E. P. Riley, and A. W. Toga, "Abnormal cortical thickness and brain–behavior correlation patterns in individuals with heavy prenatal alcohol exposure," *Cerebral Cortex,* vol. 18, no. 1, pp. 136-144, 2008.

[43] J. Joseph *et al.*, "Three-dimensional surface deformation-based shape analysis of hippocampus and caudate nucleus in children with fetal alcohol spectrum disorders," *Human Brain Mapping,* vol. 35, no. 2, pp. 659-672, 2014.

[44] F. L. Bookstein, A. P. Streissguth, P. D. Sampson, P. D. Connor, and H. M. Barr, "Corpus callosum shape and neuropsychological deficits in adult males with heavy fetal alcohol exposure," *Neuroimage,* vol. 15, no. 1, pp. 233-251, 2002.

[45] E. R. Sowell *et al.*, "Regional brain shape abnormalities persist into adolescence after heavy prenatal alcohol exposure," *Cerebral Cortex,* vol. 12, no. 8, pp. 856-865, 2002.

[46] F. De Guio *et al.*, "A study of cortical morphology in children with fetal alcohol spectrum disorders," *Human Brain Mapping,* vol. 35, no. 5, pp. 2285-2296, 2014.

[47] J. C. Culham and N. G. Kanwisher, "Neuroimaging of cognitive functions in human parietal cortex," *Current Opinion in Neurobiology,* vol. 11, no. 2, pp. 157-163, 2001.

[48] R. Stanescu-Cosson, P. Pinel, P.-F. van de Moortele, D. Le Bihan, L. Cohen, and S. Dehaene, "Understanding dissociations in dyscalculia," *Brain,* vol. 123, no. 11, pp. 2240-2255, 2000.

[49] S. Dehaene, E. Spelke, P. Pinel, R. Stanescu, and S. Tsivkin, "Sources of mathematical thinking: Behavioral and brain-imaging evidence," *Science,* vol. 284, no. 5416, pp. 970-974, 1999.

[50] S. Dehaene, M. Piazza, P. Pinel, and L. Cohen, "Three parietal circuits for number processing," *Cognitive Neuropsychology,* vol. 20, no. 3-6, pp. 487-506, 2003.

[51] M. R. Uncapher, J. B. Hutchinson, and A. D. Wagner, "A roadmap to brain mapping: toward a functional map of human parietal cortex," *Neuron,* vol. 67, no. 1, pp. 5-8, 2010.

[52] H. Garavan, T. Ross, and E. Stein, "Right hemispheric dominance of inhibitory control: an event-related functional MRI study," *Proceedings of the National Academy of Sciences,* vol. 96, no. 14, pp. 8301-8306, 1999.

[53] G. De Zubicaray, C. Andrew, F. Zelaya, S. Williams, and C. Dumanoir, "Motor response suppression and the prepotent tendency to respond: a parametric fMRI study," *Neuropsychologia,* vol. 38, no. 9, pp. 1280-1291, 2000.

[54] K. S. LaBar, D. R. Gitelman, T. B. Parrish, and M.-M. Mesulam, "Neuroanatomic overlap of working memory and spatial attention networks: a functional MRI comparison within subjects," *Neuroimage,* vol. 10, no. 6, pp. 695-704, 1999.

[55] J. B. Hutchinson, M. R. Uncapher, and A. D. Wagner, "Posterior parietal cortex and episodic retrieval: convergent and divergent effects of attention and memory," *Learning & Memory,* vol. 16, no. 6, pp. 343-356, 2009.

[56] W. Sturm *et al.*, "Functional anatomy of intrinsic alertness: evidencefor a fronto-parietal-thalamic-brainstem network in theright hemisphere," *Neuropsychologia,* vol. 37, no. 7, pp. 797-805, 1999.

[57] M.-H. Sohn, S. Ursu, J. R. Anderson, V. A. Stenger, and C. S. Carter, "The role of prefrontal cortex and posterior parietal cortex in task switching," *Proceedings of the National Academy of Sciences,* vol. 97, no. 24, pp. 13448-13453, 2000.

[58] J. Gottlieb, P. Balan, J. Oristaglio, and M. Suzuki, "Parietal control of attentional guidance: the significance of sensory, motivational and motor factors," *Neurobiology of Learning and Memory,* vol. 91, no. 2, pp. 121-128, 2009.

[59] A. Nieder and S. Dehaene, "Representation of number in the brain," *Annual Review of Neuroscience,* vol. 32, pp. 185-208, 2009.

[60] E. M. Meintjes *et al.*, "An fMRI study of number processing in children with fetal alcohol syndrome," *Alcoholism: Clinical and Experimental Research,* vol. 34, no. 8, pp. 1450-1464, 2010.

[61] D. A. Hamilton, P. Kodituwakku, R. J. Sutherland, and D. D. Savage, "Children with Fetal Alcohol Syndrome are impaired at place learning but not cued-navigation in a virtual Morris water task," *Behavioural Brain Research,* vol. 143, no. 1, pp. 85-94, 2003.

[62] J. R. Wozniak *et al.*, "Global functional connectivity abnormalities in children with fetal alcohol spectrum disorders," *Alcoholism: Clinical and Experimental Research,* vol. 37, no. 5, pp. 748-756, 2013.

[63] P. Santhanam, C. D. Coles, Z. Li, L. Li, M. E. Lynch, and X. Hu, "Default mode network dysfunction in adults with prenatal alcohol exposure," *Psychiatry Research: Neuroimaging,* vol. 194, no. 3, pp. 354-362, 2011.

[64] J. R. Wozniak *et al.*, "Inter-hemispheric functional connectivity disruption in children with prenatal alcohol exposure," *Alcoholism: Clinical and Experimental Research,* vol. 35, no. 5, pp. 849-861, 2011.

[65] F. F. Roussotte *et al.*, "Frontostriatal connectivity in children during working memory and the effects of prenatal methamphetamine, alcohol, and polydrug exposure," *Developmental Neuroscience,* vol. 34, no. 1, pp. 43-57, 2012.

[66] S. L. Fryer, S. F. Tapert, S. N. Mattson, M. P. Paulus, A. D. Spadoni, and E. P. Riley, "Prenatal alcohol exposure affects frontal–striatal BOLD response during inhibitory control," *Alcoholism: Clinical and Experimental Research,* vol. 31, no. 8, pp. 1415-1424, 2007.

[67] A. L. Ware *et al.*, "An fMRI study of behavioral response inhibition in adolescents with and without histories of heavy prenatal alcohol exposure," *Behavioural Brain Research,* vol. 278, pp. 137-146, 2015.

[68] J. W. O'Brien *et al.*, "Effect of predictive cuing on response inhibition in children with heavy prenatal alcohol exposure," *Alcoholism: Clinical and Experimental Research,* vol. 37, no. 4, pp. 644-654, 2013.

[69] Z. Li, C. D. Coles, M. E. Lynch, X. Ma, S. Peltier, and X. Hu, "Occipital-temporal reduction and sustained visual attention deficit in prenatal alcohol exposed adults," *Brain Imaging and Behavior,* vol. 2, no. 1, pp. 39-48, 2008.

[70] C. R. O'Conaill *et al.*, "Visual search for feature conjunctions: an fMRI study comparing alcohol-related neurodevelopmental disorder (ARND) to ADHD," *Journal of Neurodevelopmental Disorders,* vol. 7, no. 1, p. 1, 2015.

[71] S. J. Astley *et al.*, "Functional magnetic resonance imaging outcomes from a comprehensive magnetic resonance study of children with fetal alcohol spectrum disorders," *Journal of Neurodevelopmental Disorders,* vol. 1, no. 1, pp. 61-80, 2009.

[72] E. D. O'Hare *et al.*, "Altered frontal-parietal functioning during verbal working memory in children and adolescents with heavy prenatal alcohol exposure," *Human Brain Mapping,* vol. 30, no. 10, pp. 3200-3208, 2009.

[73] V. A. Diwadkar *et al.*, "Differences in cortico-striatal-cerebellar activation during working memory in syndromal and nonsyndromal children with prenatal alcohol exposure," *Human Brain Mapping,* vol. 34, no. 8, pp. 1931-1945, 2013.

[74] E. R. Sowell *et al.*, "Functional magnetic resonance imaging of verbal learning in children with heavy prenatal alcohol exposure," *Neuroreport,* vol. 18, no. 7, pp. 635-639, 2007.

[75] L. du Plessis *et al.*, "Neural correlates of cerebellar-mediated timing during finger tapping in children with fetal alcohol spectrum disorders," *NeuroImage: Clinical,* vol. 7, pp. 562-570, 2015.

[76] A. D. Spadoni, A. D. Bazinet, S. L. Fryer, S. F. Tapert, S. N. Mattson, and E. P. Riley, "BOLD response during spatial working memory in youth with heavy prenatal alcohol exposure," *Alcoholism: Clinical and Experimental Research,* vol. 33, no. 12, pp. 2067-2076, 2009.

[77] K. L. Malisza, A.-A. Allman, D. Shiloff, L. Jakobson, S. Longstaffe, and A. E. Chudley, "Evaluation of spatial working memory function in children and adults with fetal alcohol spectrum disorders: a functional magnetic resonance imaging study," *Pediatric Research,* vol. 58, no. 6, pp. 1150-1157, 2005.

[78] A. L. Norman *et al.*, "An fMRI study of spatial working memory in children with prenatal alcohol exposure: Contribution of familial history of alcohol use disorders," *Alcoholism: Clinical and Experimental Research,* vol. 37, no. 1, p. 132, 2013.

[79] K. L. Malisza *et al.*, "Comparison of spatial working memory in children with prenatal alcohol exposure and those diagnosed with ADHD; A functional magnetic resonance imaging study," *Journal of Neurodevelopmental Disorders,* vol. 4, no. 1, pp. 1-20, 2012.

[80] F. F. Roussotte *et al.*, "Abnormal brain activation during working memory in children with prenatal exposure to drugs of abuse: the effects of methamphetamine, alcohol, and polydrug exposure," *Neuroimage,* vol. 54, no. 4, pp. 3067-3075, 2011.

[81] P. Santhanam, Z. Li, X. Hu, M. E. Lynch, and C. D. Coles, "Effects of prenatal alcohol exposure on brain activation during an arithmetic task: an fMRI study," *Alcoholism: Clinical and Experimental Research,* vol. 33, no. 11, pp. 1901-1908, 2009.

[82] K. Woods, E. Meintjes, C. Molteno, S. Jacobson, and J. Jacobson, "Parietal dysfunction during number processing in children with fetal alcohol spectrum disorders," *NeuroImage: Clinical,* 2015.
[83] K. Kopera-Frye, S. Dehaene, and A. P. Streissguth, "Impairments of number processing induced by prenatal alcohol exposure," *Neuropsychologia,* vol. 34, no. 12, pp. 1187-1196, 1996.
[84] A. P. Streissguth, H. M. Barr, P. D. Sampson, and F. L. Bookstein, "Prenatal alcohol and offspring development: the first fourteen years," *Drug and Alcohol Dependence,* vol. 36, no. 2, pp. 89-99, 1994.
[85] S. N. Mattson *et al.*, "Toward a neurobehavioral profile of fetal alcohol spectrum disorders," *Alcoholism: Clinical and Experimental Research,* vol. 34, no. 9, pp. 1640-1650, 2010.
[86] E. Meintjes, K. Thomas, K. Woods, C. Molteno, J. Jacobson, and S. Jacobson, "Differential brain activation on a virtual water maze task by fetal alcohol-exposed children," presented at the Organization for Human Brain Mapping, 2012.
[87] H. Gray, *Anatomy of the human body*. Lea & Febiger, 1918.
[88] S. L. Fryer *et al.*, "Characterization of white matter microstructure in fetal alcohol spectrum disorders," *Alcoholism: Clinical and Experimental Research,* vol. 33, no. 3, pp. 514-521, 2009.
[89] E. R. Sowell *et al.*, "Mapping white matter integrity and neurobehavioral correlates in children with fetal alcohol spectrum disorders," *Journal of Neuroscience,* vol. 28, no. 6, pp. 1313-1319, 2008.
[90] C. Lebel, C. Rasmussen, K. Wyper, G. Andrew, and C. Beaulieu, "Brain microstructure is related to math ability in children with fetal alcohol spectrum disorder," *Alcoholism: Clinical and Experimental Research,* vol. 34, no. 2, pp. 354-363, 2010.
[91] S. Jacobson, N. Dodge, S. Dehaene, L. Chiodo, R. Sokol, and J. Jacobson, "Evidence for a specific effect of prenatal alcohol exposure on 'number sense'," *Alcoholism: Clinical and Experimental Research,* vol. 27, p. A121, 2003.
[92] A. Uecker and L. Nadel, "Spatial locations gone awry: object and spatial memory deficits in children with fetal alcohol syndrome," *Neuropsychologia,* vol. 34, no. 3, pp. 209-223, 1996.
[93] A. Uecker and L. Nadel, "Spatial but not object memory impairments in children with fetal alcohol syndrome," *American Journal on Mental Retardation,* vol. 103, no. 1, pp. 12-18, 1998.
[94] K. L. Kaemingk and P. Tanner Halverson, "Spatial memory following prenatal alcohol exposure: More than a material specific memory deficit," *Child Neuropsychology,* vol. 6, no. 2, pp. 115-128, 2000.
[95] K. L. Kaemingk, S. Mulvaney, and P. T. Halverson, "Learning following prenatal alcohol exposure: performance on verbal and visual multitrial tasks," *Archives of Clinical Neuropsychology,* vol. 18, no. 1, pp. 33-47, 2003.
[96] A. L. Norman *et al.*, "A functional magnetic resonance imaging study of spatial working memory in children with prenatal alcohol exposure: contribution of familial history of alcohol use disorders," *Alcoholism: Clinical and Experimental Research,* vol. 37, no. 1, pp. 132-140, 2013.
[97] C. Green *et al.*, "Executive function deficits in children with fetal alcohol spectrum disorders (FASD) measured using the Cambridge Neuropsychological Tests Automated Battery (CANTAB)," *Journal of Child Psychology and Psychiatry,* vol. 50, no. 6, pp. 688-697, 2009.
[98] C. D. Coles, M. E. Lynch, J. A. Kable, K. C. Johnson, and F. C. Goldstein, "Verbal and nonverbal memory in adults prenatally exposed to alcohol," *Alcoholism: Clinical and Experimental Research,* vol. 34, no. 5, pp. 897-906, 2010.
[99] D. Sheslow and W. Adams, *WRAML: Wide range assessment of memory and learning*. Adams and Sheslow, 1990.
[100] B. A. Blanchard, E. Riley, and J. H. Hannigan, "Deficits on a spatial navigation task following prenatal exposure to ethanol," *Neurotoxicology and Teratology,* vol. 9, no. 3, pp. 253-258, 1987.
[101] C. Gianoulakis, "Rats exposed prenatally to alcohol exhibit impairment in spatial navigation test," *Behavioural Brain Research,* vol. 36, no. 3, pp. 217-228, 1990.
[102] D. Richardson, M. Byrnes, J. Brien, J. Reynolds, and H. Dringenberg, "Impaired acquisition in the water maze and hippocampal long-term potentiation after chronic prenatal ethanol exposure in the guinea-pig," *European Journal of Neuroscience,* vol. 16, no. 8, pp. 1593-1598, 2002.
[103] R. Morris, "Developments of a water-maze procedure for studying spatial learning in the rat," *Journal of Neuroscience Methods,* vol. 11, no. 1, pp. 47-60, 1984.
[104] L. Nadel, "The hippocampus and space revisited," *Hippocampus,* vol. 1, no. 3, pp. 221-229, 1991.
[105] R. S. Astur, M. L. Ortiz, and R. J. Sutherland, "A characterization of performance by men and women in a virtual Morris water task: A large and reliable sex difference," *Behavioural Brain Research,* vol. 93, no. 1, pp. 185-190, 1998.

[106] W. J. Jacobs, H. E. Laurance, and K. G. Thomas, "Place learning in virtual space I: Acquisition, overshadowing, and transfer," *Learning and Motivation,* vol. 28, no. 4, pp. 521-541, 1997.
[107] W. J. Jacobs, K. G. Thomas, H. E. Laurance, and L. Nadel, "Place learning in virtual space: II. Topographical relations as one dimension of stimulus control," *Learning and Motivation,* vol. 29, no. 3, pp. 288-308, 1998.
[108] K. G. Thomas, M. Hsu, H. E. Laurance, L. Nadel, and W. J. Jacobs, "Place learning in virtual space III: Investigation of spatial navigation training procedures and their application to fMRI and clinical neuropsychology," *Behavior Research Methods, Instruments, & Computers,* vol. 33, no. 1, pp. 21-37, 2001.
[109] S. J. Astley and S. K. Clarren, "Measuring the facial phenotype of individuals with prenatal alcohol exposure: correlations with brain dysfunction," *Alcohol and Alcoholism,* vol. 36, no. 2, pp. 147-159, 2001.
[110] P. Jezzard, P. M. Matthews, and S. M. Smith, *Functional MRI: an introduction to methods*. Oxford University Press, 2001.
[111] N. K. Logothetis, "What we can do and what we cannot do with fMRI," *Nature,* vol. 453, no. 7197, pp. 869-878, 2008.
[112] C. D. Coles and Z. Li, "Functional neuroimaging in the examination of effects of prenatal alcohol exposure," *Neuropsychology Review,* vol. 21, no. 2, pp. 119-132, 2011.
[113] S. Ogawa *et al.*, "Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging," *Proceedings of the National Academy of Sciences,* vol. 89, no. 13, pp. 5951-5955, 1992.
[114] P. M. Matthews and P. Jezzard, "Functional magnetic resonance imaging," *Journal of Neurology, Neurosurgery & Psychiatry,* vol. 75, no. 1, pp. 6-12, 2004.
[115] J. C. Gore, "Principles and practice of functional MRI of the human brain," *The Journal of Clinical Investigation,* vol. 112, no. 1, pp. 4-9, 2003.
[116] E. Amaro and G. J. Barker, "Study design in fMRI: basic principles," *Brain and Cognition,* vol. 60, no. 3, pp. 220-232, 2006.
[117] M. A. Lindquist, "The statistical analysis of fMRI data," *Statistical Science,* vol. 23, no. 4, pp. 439-464, 2008.
[118] R. Goebel, F. Esposito, and E. Formisano, "Analysis of functional image analysis contest (FIAC) data with brainvoyager QX: From single-subject to cortically aligned group general linear model analysis and self-organizing group independent component analysis," *Human Brain Mapping,* vol. 27, no. 5, pp. 392-401, 2006.
[119] J. Talairach and P. Tournoux, "Co-planar stereotaxic atlas of the human brain. 3-Dimensional proportional system: an approach to cerebral imaging," 1988.
[120] K. J. Friston, A. Holmes, J.-B. Poline, C. J. Price, and C. D. Frith, "Detecting activations in PET and fMRI: levels of inference and power," *Neuroimage,* vol. 4, no. 3, pp. 223-235, 1996.
[121] K. J. Worsley, A. C. Evans, S. Marrett, and P. Neelin, "A three-dimensional statistical analysis for CBF activation studies in human brain," *Journal of Cerebral Blood Flow & Metabolism,* vol. 12, no. 6, pp. 900-918, 1992.
[122] S. D. Forman, J. D. Cohen, M. Fitzgerald, W. F. Eddy, M. A. Mintun, and D. C. Noll, "Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold," *Magnetic Resonance in Medicine,* vol. 33, no. 5, pp. 636-647, 1995.
[123] E. P. Riley and C. L. McGee, "Fetal alcohol spectrum disorders: an overview with emphasis on changes in brain and behavior," *Experimental Biology and Medicine,* vol. 230, no. 6, pp. 357-365, 2005.
[124] C. D. Coles, K. A. Platzman, C. L. Raskind-Hood, R. T. Brown, A. Falek, and I. E. Smith, "A comparison of children affected by prenatal alcohol exposure and attention deficit, hyperactivity disorder," *Alcoholism: Clinical and Experimental Research,* vol. 21, no. 1, pp. 150-161, 1997.
[125] M. J. Burden, S. W. Jacobson, R. J. Sokol, and J. L. Jacobson, "Effects of prenatal alcohol exposure on attention and working memory at 7.5 years of age," *Alcoholism: Clinical and Experimental Research,* vol. 29, no. 3, pp. 443-452, 2005.
[126] S. W. Jacobson, J. L. Jacobson, M. E. Stanton, E. M. Meintjes, and C. D. Molteno, "Biobehavioral markers of adverse effect in fetal alcohol spectrum disorders," *Neuropsychology Review,* vol. 21, no. 2, pp. 148-166, 2011.
[127] A. P. Streissguth, J. M. Aase, S. K. Clarren, S. P. Randels, R. A. LaDue, and D. F. Smith, "Fetal alcohol syndrome in adolescents and adults," *JAMA: The Journal of the American Medical Association,* vol. 265, no. 15, pp. 1961-1967, 1991.

[128] L. Goldschmidt, G. A. Richardson, D. S. Stoffer, D. Geva, and N. L. Day, "Prenatal alcohol exposure and academic achievement at age six: a nonlinear fit," *Alcoholism: Clinical and Experimental Research,* vol. 20, no. 4, pp. 763-770, 1996.

[129] K. K. Howell, M. E. Lynch, K. A. Platzman, G. H. Smith, and C. D. Coles, "Prenatal alcohol exposure and ability, academic achievement, and school functioning in adolescence: a longitudinal follow-up," *Journal of Pediatric Psychology,* vol. 31, no. 1, pp. 116-126, 2006.

[130] K. A. Kerns, A. Don, C. A. Mateer, and A. P. Streissguth, "Cognitive deficits in nonretarded adults with fetal alcohol syndrome," *Journal of Learning Disabilities,* vol. 30, no. 6, pp. 685-693, 1997.

[131] S. Henschen, "Über sprach-, musik-und rechenmechanismen und ihre lokalisationen im großhirn," *Zeitschrift für die Gesamte Neurologie und Psychiatrie,* vol. 52, no. 1, pp. 273-298, 1919.

[132] S. Dehaene, "Varieties of numerical abilities," *Cognition,* vol. 44, no. 1, pp. 1-42, 1992.

[133] S. Dehaene and L. Cohen, "Towards an anatomical and functional model of number processing," *Mathematical Cognition,* vol. 1, pp. 83-120, 1996.

[134] S. Rivera, A. Reiss, M. Eckert, and V. Menon, "Developmental changes in mental arithmetic: evidence for increased functional specialization in the left inferior parietal cortex," *Cerebral Cortex,* vol. 15, no. 11, pp. 1779-1790, 2005.

[135] L. Kaufmann, G. Wood, O. Rubinsten, and A. Henik, "Meta-analyses of developmental fMRI studies investigating typical and atypical trajectories of number processing and calculation," *Developmental Neuropsychology,* vol. 36, no. 6, pp. 763-787, 2011.

[136] E. M. Meintjes *et al.*, "An fMRI study of magnitude comparison and exact addition in children," *Magnetic Resonance Imaging,* vol. 28, no. 3, pp. 351-362, 2010.

[137] M. Suttie *et al.*, "Facial dysmorphism across the fetal alcohol spectrum," *Pediatrics,* vol. 131, no. 3, pp. e779-e788, 2013.

[138] E. Meintjes *et al.*, "Tensor-based morphometry reveals brain midline tissue reduction in children with heavy prenatal alcohol exposure," in *Alcoholism: Clinical and Experimental Research*, 2013, vol. 37, pp. 105A-105A: WILEY-BLACKWELL 111 RIVER ST, HOBOKEN 07030-5774, NJ USA.

[139] L. du Plessis *et al.*, "Neurochemical Effects of Fetal Alcohol Exposure in the Deep Cerebellar Nuclei," presented at the Organization for Human Brain Mapping, 2012.

[140] S. W. Jacobson *et al.*, "Impaired delay and trace eyeblink conditioning in school-age children with fetal alcohol syndrome," *Alcoholism: Clinical and Experimental Research,* vol. 35, no. 2, pp. 250-264, 2011.

[141] S. W. Jacobson *et al.*, "Impaired eyeblink conditioning in children with fetal alcohol syndrome," *Alcoholism: Clinical and Experimental Research,* vol. 32, no. 2, pp. 365-372, 2008.

[142] S. W. Jacobson, L. M. Chiodo, R. J. Sokol, and J. L. Jacobson, "Validity of maternal report of prenatal alcohol, cocaine, and smoking in relation to neurobehavioral outcome," *Pediatrics,* vol. 109, no. 5, pp. 815-825, 2002.

[143] R. J. Sokol, S. Debanne, J. Ager, S. Martier, C. Ernhart, and L. Chik, "Maximizing the efficiency of a study on alcohol-related birth defects by means of data collection and analytic strategy dissociation," *American Journal of Perinatology,* vol. 2, no. 03, pp. 245-249, 1985.

[144] R. S. Bowman, L. I. Stein, and J. R. Newton, "Measurement and interpretation of drinking behavior," *Journal of Studies on Alcohol and Drugs,* vol. 36, no. 09, p. 1154, 1975.

[145] J. M. Sattler, *Assessment of children 3rd edition*. San Diego: Jerome M. Sattler, Inc, 1992.

[146] B. P. Lanphear, K. Dietrich, P. Auinger, and C. Cox, "Cognitive deficits associated with blood lead concentrations< 10 microg/dL in US children and adolescents," *Public Health Reports,* vol. 115, no. 6, p. 521, 2000.

[147] E. Eger, P. Sterzer, M. O. Russ, A.-L. Giraud, and A. Kleinschmidt, "A supramodal number representation in human intraparietal cortex," *Neuron,* vol. 37, no. 4, pp. 719-726, 2003.

[148] F. Chochon, L. Cohen, P. Van De Moortele, and S. Dehaene, "Differential contributions of the left and right inferior parietal lobules to number processing," *Journal of Cognitive Neuroscience,* vol. 11, no. 6, pp. 617-630, 1999.

[149] M. Pesenti, M. Thioux, X. Seron, and A. De Volder, "Neuroanatomical substrates of Arabic number processing, numerical comparison, and simple addition: A PET study," *Journal of Cognitive Neuroscience,* vol. 12, no. 3, pp. 461-479, 2000.

[150] P. Burbaud, O. Camus, D. Guehl, B. Bioulac, J.-M. Caillé, and M. Allard, "A functional magnetic resonance imaging study of mental subtraction in human subjects," *Neuroscience Letters,* vol. 273, no. 3, pp. 195-199, 1999.
[151] P. Pinel, S. Dehaene, D. Riviere, and D. LeBihan, "Modulation of parietal activation by semantic distance in a number comparison task," *Neuroimage,* vol. 14, no. 5, pp. 1013-1026, 2001.
[152] D. W. Langdon and E. K. Warrington, "The abstraction of numerical relations: a role for the right hemisphere in arithmetic?," *Journal of the International Neuropsychological Society,* vol. 3, pp. 260-268, 1997.
[153] M. Rosselli and A. Ardila, "Calculation deficits in patients with right and left hemisphere damage," *Neuropsychologia,* vol. 27, no. 5, pp. 607-617, 1989.
[154] M. Kiefer and S. Dehaene, "The time course of parietal activation in single-digit multiplication: Evidence from event-related potentials," *Mathematical Cognition,* vol. 3, no. 1, pp. 1-30, 1997.
[155] R. Stanescu-Cosson, P. Pinel, P. Van de Moortele, D. Le Bihan, L. Cohen, and S. Dehaene, "Cerebral bases of calculation processes: Impact of number size on the cerebral circuits for exact and approximate calculation," *Brain,* vol. 123, pp. 2240-2255, 2000.
[156] S. M. Rivera, V. Menon, C. D. White, B. Glaser, and A. L. Reiss, "Functional brain activation during arithmetic processing in females with fragile X Syndrome is related to FMR1 protein expression," *Human Brain Mapping,* vol. 16, no. 4, pp. 206-218, 2002.
[157] R. S. Moyer and T. K. Landauer, "Time required for judgements of numerical inequality," 1967.
[158] L. du Plessis *et al.*, "An in vivo 1H magnetic resonance spectroscopy study of the deep cerebellar nuclei in children with fetal alcohol spectrum disorders," *Alcoholism: Clinical and Experimental Research,* vol. 38, no. 5, pp. 1330-1338, 2014.
[159] K. Kucian, T. Loenneker, T. Dietrich, M. Dosch, E. Martin, and M. von Aster, "Impaired neural networks for approximate calculation in dyscalculic children: a functional MRI study," *Behavioral and Brain Functions,* vol. 2, no. 31, pp. 1-17, 2006.
[160] L. Kaufmann, S. Vogel, M. Starke, C. Kremser, and M. Schocke, "Numerical and non-numerical ordinality processing in children with and without developmental dyscalculia: Evidence from fMRI," *Cognitive Development,* vol. 24, no. 4, pp. 486-494, 2009.
[161] L. Kaufmann, S. E. Vogel, M. Starke, C. Kremser, M. Schocke, and G. Wood, "Developmental dyscalculia: compensatory mechanisms in left intraparietal regions in response to nonsymbolic magnitudes," *Behavioral and Brain Functions,* vol. 5, no. 1, p. 35, 2009.
[162] S. Ashkenazi, M. Rosenberg-Lee, C. Tenison, and V. Menon, "Weak task-related modulation and stimulus representations during arithmetic problem solving in children with developmental dyscalculia," *Developmental Cognitive Neuroscience,* vol. 2, pp. S152-S166, 2012.
[163] G. R. Price, I. Holloway, P. Räsänen, M. Vesterinen, and D. Ansari, "Impaired parietal magnitude processing in developmental dyscalculia," *Current Biology,* vol. 17, no. 24, pp. R1042-R1043, 2007.
[164] C. Mussolin, A. De Volder, C. Grandin, X. Schlögel, M.-C. Nassogne, and M.-P. Noël, "Neural correlates of symbolic number comparison in developmental dyscalculia," *Journal of Cognitive Neuroscience,* vol. 22, no. 5, pp. 860-874, 2010.
[165] O. Rubinsten and A. Henik, "Developmental dyscalculia: heterogeneity might not mean different mechanisms," *Trends in Cognitive Sciences,* vol. 13, no. 2, pp. 92-99, 2009.
[166] B. De Smedt, I. D. Holloway, and D. Ansari, "Effects of problem size and arithmetic operation on brain activation during calculation in children with varying levels of arithmetical fluency," *Neuroimage,* vol. 57, no. 3, pp. 771-781, 2011.
[167] E. Isaacs, C. Edmonds, A. Lucas, and D. Gadian, "Calculation difficulties in children of very low birthweight A neural correlate," *Brain,* vol. 124, no. 9, pp. 1701-1707, 2001.
[168] N. Molko *et al.*, "Functional and structural alterations of the intraparietal sulcus in a developmental dyscalculia of genetic origin," *Neuron,* vol. 40, no. 4, pp. 847-858, 2003.
[169] M. Delazer *et al.*, "Learning by strategies and learning by drill—Evidence from an fMRI study," *Neuroimage,* vol. 25, no. 3, pp. 838-849, 2005.
[170] O. Simon, J.-F. Mangin, L. Cohen, D. Le Bihan, and S. Dehaene, "Topographical layout of hand, eye, calculation, and language-related areas in the human parietal lobe," *Neuron,* vol. 33, no. 3, pp. 475-487, 2002.

[171] D. C. Geary, S. C. Brown, and V. Samaranayake, "Cognitive addition: A short longitudinal study of strategy choice and speed-of-processing differences in normal and mathematically disabled children," *Developmental psychology,* vol. 27, no. 5, p. 787, 1991.
[172] M. H. Ashcraft, "The development of mental arithmetic: A chronometric approach," *Developmental Review,* vol. 2, no. 3, pp. 213-236, 1982.
[173] M. Piazza, A. Mechelli, B. Butterworth, and C. J. Price, "Are subitizing and counting implemented as separate or functionally overlapping processes?," *Neuroimage,* vol. 15, no. 2, pp. 435-446, 2002.
[174] S. M. Ravizza, M. R. Delgado, J. M. Chein, J. T. Becker, and J. A. Fiez, "Functional dissociations within the inferior parietal cortex in verbal working memory," *Neuroimage,* vol. 22, no. 2, pp. 562-573, 2004.
[175] P. Gautam *et al.*, "Developmental trajectories for visuo-spatial attention are altered by prenatal alcohol exposure: A longitudinal fMRI study," *Cerebral Cortex,* p. 162, 2014.
[176] L. Kaufmann *et al.*, "Neural correlates of distance and congruity effects in a numerical Stroop task: an event-related fMRI study," *Neuroimage,* vol. 25, no. 3, pp. 888-898, 2005.
[177] D. Ansari and B. Dhital, "Age-related changes in the activation of the intraparietal sulcus during nonsymbolic magnitude processing: an event-related functional magnetic resonance imaging study," *Journal of Cognitive Neuroscience,* vol. 18, no. 11, pp. 1820-1828, 2006.
[178] C. Mussolin, S. Mejias, and M.-P. Noël, "Symbolic and nonsymbolic number comparison in children with and without dyscalculia," *Cognition,* vol. 115, no. 1, pp. 10-25, 2010.
[179] M. M. Gullick, L. A. Sprute, and E. Temple, "Individual differences in working memory, nonverbal IQ, and mathematics achievement and brain mechanisms associated with symbolic and nonsymbolic number processing," *Learning and Individual Differences,* vol. 21, no. 6, pp. 644-654, 2011.
[180] A. B. Hollingshead, "Four factor index of social status," *Yale Journal of Sociology,* no. 8, pp. 21-51, 2011.
[181] G. Le Clec'H *et al.*, "Distinct cortical areas for names of numbers and body parts independent of language and input modality," *Neuroimage,* vol. 12, no. 4, pp. 381-391, 2000.
[182] K. M. Lee, "Cortical areas differentially involved in multiplication and subtraction: a functional magnetic resonance imaging study and correlation with a case of selective acalculia," *Annals of Neurology,* vol. 48, no. 4, pp. 657-661, 2000.
[183] L. Kaufmann *et al.*, "Neural correlates of the number–size interference task in children," *Neuroreport,* vol. 17, no. 6, p. 587, 2006.
[184] I. D. Holloway and D. Ansari, "Mapping numerical magnitudes onto symbols: The numerical distance effect and individual differences in children's mathematics achievement," *Journal of Experimental Child Psychology,* vol. 103, no. 1, pp. 17-29, 2009.
[185] D. Ansari, B. Dhital, and S. C. Siong, "Parametric effects of numerical distance on the intraparietal sulcus during passive viewing of rapid numerosity changes," *Brain Research,* vol. 1067, no. 1, pp. 181-188, 2006.
[186] J. Lonnemann, J. Linkersdörfer, M. Hasselhorn, and S. Lindberg, "Symbolic and non-symbolic distance effects in children and their connection with arithmetic skills," *Journal of Neurolinguistics,* vol. 24, no. 5, pp. 583-591, 2011.
[187] K. Kucian, T. Loenneker, E. Martin, and M. von Aster, "Non-symbolic numerical distance effect in children with and without developmental dyscalculia: a parametric fMRI study," *Developmental Neuropsychology,* vol. 36, no. 6, pp. 741-762, 2011.
[188] I. D. Holloway, G. R. Price, and D. Ansari, "Common and segregated neural pathways for the processing of symbolic and nonsymbolic numerical magnitude: an fMRI study," *Neuroimage,* vol. 49, no. 1, pp. 1006-1017, 2010.
[189] G. R. Price and D. Ansari, "Symbol processing in the left angular gyrus: evidence from passive perception of digits," *Neuroimage,* vol. 57, no. 3, pp. 1205-1211, 2011.
[190] V. Menon, S. Rivera, C. White, G. Glover, and A. Reiss, "Dissociating prefrontal and parietal cortex activation during arithmetic processing," *Neuroimage,* vol. 12, no. 4, pp. 357-365, 2000.
[191] R. H. Grabner, D. Ansari, G. Reishofer, E. Stern, F. Ebner, and C. Neuper, "Individual differences in mathematical competence predict parietal brain activation during mental calculation," *Neuroimage,* vol. 38, no. 2, pp. 346-356, 2007.

[192] D. Ansari, "Effects of development and enculturation on number representation in the brain," *Nature Reviews Neuroscience,* vol. 9, no. 4, pp. 278-291, 2008.
[193] A. Nobre, G. Sebestyen, D. Gitelman, M. Mesulam, R. Frackowiak, and C. Frith, "Functional localization of the system for visuospatial attention using positron emission tomography," *Brain,* vol. 120, no. 3, pp. 515-533, 1997.
[194] D. R. Gitelman *et al.*, "A large-scale distributed network for covert spatial attention," *Brain,* vol. 122, no. 6, pp. 1093-1106, 1999.
[195] M. Corbetta and G. L. Shulman, "Human cortical mechanisms of visual attention during orienting and search," *Philosophical Transactions of the Royal Society of London B: Biological Sciences,* vol. 353, no. 1373, pp. 1353-1362, 1998.
[196] M. Corbetta, J. M. Kincade, J. M. Ollinger, M. P. McAvoy, and G. L. Shulman, "Voluntary orienting is dissociated from target detection in human posterior parietal cortex," *Nature Neuroscience,* vol. 3, no. 3, pp. 292-297, 2000.
[197] M. Rushworth, M. Krams, and R. Passingham, "Complementary lateralization of attention and intention in the human brain. A PET study," *Journal of Cognitive Neuroscience,* vol. 13, pp. 698-710, 2001.
[198] M. F. Rushworth, T. Paus, and P. K. Sipila, "Attention systems and the organization of the human parietal cortex," *The Journal of Neuroscience,* vol. 21, no. 14, pp. 5262-5271, 2001.
[199] S. Göbel, V. Walsh, and M. F. Rushworth, "The mental number line and the human angular gyrus," *Neuroimage,* vol. 14, no. 6, pp. 1278-1289, 2001.
[200] M. L. Seghier, "The angular gyrus multiple functions and multiple subdivisions," *The Neuroscientist,* vol. 19, no. 1, pp. 43-61, 2013.
[201] R. L. Buckner, J. R. Andrews-Hanna, and D. L. Schacter, "The brain's default network," *Annals of the New York Academy of Sciences,* vol. 1124, no. 1, pp. 1-38, 2008.
[202] K. A. Mckiernan, J. N. Kaufman, J. Kucera-Thompson, and J. R. Binder, "A parametric manipulation of factors affecting task-induced deactivation in functional neuroimaging," *Journal of Cognitive Neuroscience,* vol. 15, no. 3, pp. 394-408, 2003.
[203] J. Damoiseaux *et al.*, "Consistent resting-state networks across healthy subjects," *Proceedings of the National Academy of Sciences,* vol. 103, no. 37, pp. 13848-13853, 2006.
[204] J. Fan *et al.*, "White matter deficits mediate effects of prenatal alcohol exposure on cognitive development in childhood," *Human Brain Mapping,* 2016.
[205] J. Fan *et al.*, "Localized reductions in resting-state functional connectivity in children with prenatal alcohol exposure," *Human Brain Mapping,* 2017.
[206] R. C. Kadosh, J. Lammertyn, and V. Izard, "Are numbers special? An overview of chronometric, neuroimaging, developmental and comparative studies of magnitude representation," *Progress in Neurobiology,* vol. 84, no. 2, pp. 132-147, 2008.
[207] C. Rasmussen, "Executive functioning and working memory in fetal alcohol spectrum disorder," *Alcoholism: Clinical and Experimental Research,* vol. 29, no. 8, pp. 1359-1367, 2005.
[208] N. Crocker, L. Vaurio, E. P. Riley, and S. N. Mattson, "Comparison of verbal learning and memory in children with heavy prenatal alcohol exposure or attention-deficit/hyperactivity disorder," *Alcoholism: Clinical and Experimental Research,* vol. 35, no. 6, pp. 1114-1121, 2011.
[209] C. E. Lewis *et al.*, "Verbal learning and memory impairment in children with fetal alcohol spectrum disorders," *Alcoholism: Clinical and Experimental Research,* vol. 39, no. 4, pp. 724-732, 2015.
[210] L. Vaurio, E. P. Riley, and S. N. Mattson, "Neuropsychological comparison of children with heavy prenatal alcohol exposure and an IQ-matched comparison group," *Journal of the International Neuropsychological Society,* vol. 17, no. 03, pp. 463-473, 2011.
[211] T. M. Roebuck-Spencer and S. N. Mattson, "Implicit strategy affects learning in children with heavy prenatal alcohol exposure," *Alcoholism: Clinical and Experimental Research,* vol. 28, no. 9, pp. 1424-1431, 2004.
[212] C. Rasmussen, K. Horne, and A. Witol, "Neurobehavioral functioning in children with fetal alcohol spectrum disorder," *Child Neuropsychology,* vol. 12, no. 6, pp. 453-468, 2006.
[213] S. J. Astley *et al.*, "Neuropsychological and behavioral outcomes from a comprehensive magnetic resonance study of children with fetal alcohol spectrum disorders," *The Canadian Journal of Clinical Pharmacology = Journal Canadien de Pharmacologie Clinique,* vol. 16, no. 1, p. e178, 2009.

[214] S. N. Mattson and E. P. Riley, "A review of the neurobehavioral deficits in children with fetal alcohol syndrome or prenatal exposure to alcohol," *Alcoholism: Clinical and Experimental Research,* vol. 22, no. 2, pp. 279-294, 1998.
[215] R. G. Morris, "Spatial localization does not require the presence of local cues," *Learning and Motivation,* vol. 12, no. 2, pp. 239-260, 1981.
[216] E. Meintjes, K. Thomas, K. Woods, C. Molteno, J. Jacobson, and S. Jacobson, "Differential brain activation on a virtual water maze task by fetal alcohol-exposed children," *OHBM Conference,* 2012.
[217] E. A. Maguire, N. Burgess, and J. O'Keefe, "Human spatial navigation: cognitive maps, sexual dimorphism, and neural substrates," *Current Opinion in Neurobiology,* vol. 9, no. 2, pp. 171-177, 1999.
[218] A. L. Shelton and J. D. Gabrieli, "Neural correlates of encoding space from route and survey perspectives," *The Journal of Neuroscience,* vol. 22, no. 7, pp. 2711-2717, 2002.
[219] N. Burgess, E. A. Maguire, and J. O'Keefe, "The Human Hippocampus and Spatial and Episodic Memory," *Neuron,* vol. 35, no. 4, pp. 625-641, 2002.
[220] B. McNaughton, L. Chen, and E. Markus, "'"Dead reckoning," landmark learning, and the sense of direction: a neurophysiological and computational hypothesis," *Journal of Cognitive Neuroscience,* vol. 3, no. 2, pp. 190-202, 1991.
[221] C. Constantinidis and X.-J. Wang, "A neural circuit basis for spatial working memory," *The Neuroscientist,* vol. 10, no. 6, pp. 553-565, 2004.
[222] C. Curtis, "Prefrontal and parietal contributions to spatial working memory," *Neuroscience,* vol. 139, no. 1, pp. 173-180, 2006.
[223] E. Ricciardi, D. Bonino, C. Gentili, L. Sani, P. Pietrini, and T. Vecchi, "Neural correlates of spatial working memory in humans: a functional magnetic resonance imaging study comparing visual and tactile processes," *Neuroscience,* vol. 139, no. 1, pp. 339-349, 2006.
[224] D. S. Pine *et al.*, "Neurodevelopmental aspects of spatial navigation: a virtual reality fMRI study," *Neuroimage,* vol. 15, no. 2, pp. 396-406, 2002.
[225] H. J. Spiers and C. Barry, "Neural systems supporting navigation," *Current Opinion in Behavioral Sciences,* vol. 1, pp. 47-55, 2015.
[226] G. Grön, A. P. Wunderlich, M. Spitzer, R. Tomczak, and M. W. Riepe, "Brain activation during human navigation: gender-different neural networks as substrate of performance," *Nature Neuroscience,* vol. 3, no. 4, pp. 404-408, 2000.
[227] P. W. Halligan, G. R. Fink, J. C. Marshall, and G. Vallar, "Spatial cognition: evidence from visual neglect," *Trends in Cognitive Sciences,* vol. 7, no. 3, pp. 125-133, 2003.
[228] K. Jordan, H.-J. Heinze, K. Lutz, M. Kanowski, and L. Jäncke, "Cortical activations during the mental rotation of different visual objects," *Neuroimage,* vol. 13, no. 1, pp. 143-152, 2001.
[229] K. Jordan, T. Wüstenberg, H.-J. Heinze, M. Peters, and L. Jäncke, "Women and men exhibit different cortical activation patterns during mental rotation tasks," *Neuropsychologia,* vol. 40, no. 13, pp. 2397-2408, 2002.
[230] C. Lamm, C. Windischberger, U. Leodolter, E. Moser, and H. Bauer, "Evidence for premotor cortex activity during dynamic visuospatial imagery from single-trial functional magnetic resonance imaging and event-related slow cortical potentials," *Neuroimage,* vol. 14, no. 2, pp. 268-283, 2001.
[231] T. Thomsen *et al.*, "Functional magnetic resonance imaging (fMRI) study of sex differences in a mental rotation task," *Medical Science Monitor,* vol. 6, no. 6, pp. 1186-1196, 2000.
[232] R. Morris, P. Garrud, J. Rawlins, and J. O'Keefe, "Place navigation impaired in rats with hippocampal lesions," *Nature,* vol. 297, no. 5868, pp. 681-683, 1982.
[233] R. Morris, J. Hagan, and J. Rawlins, "Allocentric spatial learning by hippocampectomised rats: a further test of the "spatial mapping" and "working memory" theories of hippocampal function," *The Quarterly Journal of Experimental Psychology,* vol. 38, no. 4, pp. 365-395, 1986.
[234] R. Brandeis, Y. Brandys, and S. Yehuda, "The use of the Morris water maze in the study of memory and learning," *International Journal of Neuroscience,* vol. 48, no. 1-2, pp. 29-69, 1989.
[235] R. S. Astur, L. B. Taylor, A. N. Mamelak, L. Philpott, and R. J. Sutherland, "Humans with hippocampus damage display severe spatial memory impairments in a virtual Morris water task," *Behavioural Brain Research,* vol. 132, no. 1, pp. 77-84, 2002.

[236] H. J. Spiers *et al.*, "Unilateral temporal lobectomy patients show lateralized topographical and episodic memory deficits in a virtual town," *Brain,* vol. 124, no. 12, pp. 2476-2489, 2001.
[237] V. D. Bohbot, G. Iaria, and M. Petrides, "Hippocampal function and spatial memory: evidence from functional neuroimaging in healthy participants and performance of patients with medial temporal lobe resections," *Neuropsychology,* vol. 18, no. 3, p. 418, 2004.
[238] D. M. Parslow *et al.*, "Allocentric spatial memory activation of the hippocampal formation measured with fMRI," *Neuropsychology,* vol. 18, no. 3, p. 450, 2004.
[239] S. L. Shipman and R. S. Astur, "Factors affecting the hippocampal BOLD response during spatial memory," *Behavioural Brain Research,* vol. 187, no. 2, pp. 433-441, 2008.
[240] R. S. Astur, J. Tropp, S. Sava, R. T. Constable, and E. J. Markus, "Sex differences and correlations in a virtual Morris water task, a virtual radial arm maze, and mental rotation," *Behavioural Brain Research,* vol. 151, no. 1, pp. 103-115, 2004.
[241] J. T. Sneider, D. A. Hamilton, J. E. Cohen-Gilbert, D. J. Crowley, I. M. Rosso, and M. M. Silveri, "Sex differences in spatial navigation and perception in human adolescents and emerging adults," *Behavioural Processes,* vol. 111, pp. 42-50, 2015.
[242] N. T. Nowak, M. P. Diamond, S. J. Land, and S. D. Moffat, "Contributions of sex, testosterone, and androgen receptor CAG repeat number to virtual Morris water maze performance," *Psychoneuroendocrinology,* vol. 41, pp. 13-22, 2014.
[243] J. Burkitt, D. Widman, and D. M. Saucier, "Evidence for the influence of testosterone in the performance of spatial navigation in a virtual water maze in women but not in men," *Hormones and Behavior,* vol. 51, no. 5, pp. 649-654, 2007.
[244] I. Driscoll, D. A. Hamilton, R. A. Yeo, W. M. Brooks, and R. J. Sutherland, "Virtual navigation in humans: the impact of age, sex, and hormones on place learning," *Hormones and Behavior,* vol. 47, no. 3, pp. 326-335, 2005.
[245] S. C. Mueller, C. P. Jackson, and R. W. Skelton, "Sex differences in a virtual water maze: An eye tracking and pupillometry study," *Behavioural Brain Research,* vol. 193, no. 2, pp. 209-215, 2008.
[246] D. G. Woolley *et al.*, "Sex differences in human virtual water maze performance: Novel measures reveal the relative contribution of directional responding and spatial knowledge," *Behavioural Brain Research,* vol. 208, no. 2, pp. 408-414, 2010.
[247] D. J. van Gerven, A. N. Schneider, D. M. Wuitchik, and R. W. Skelton, "Direct measurement of spontaneous strategy selection in a virtual morris water maze shows females choose an allocentric strategy at least as often as males do," *Behavioral Neuroscience,* vol. 126, no. 3, p. 465, 2012.
[248] P. Newhouse, C. Newhouse, and R. S. Astur, "Sex differences in visual-spatial learning using a virtual water maze in pre-pubertal children," *Behavioural Brain Research,* vol. 183, no. 1, pp. 1-7, 2007.
[249] A. Wunderlich, "Cortical Regions Involved in Navigation," *Proceedings of the International Society for Magnetic Resonance in Medicine,* vol. 9, 2001.
[250] J. T. Sneider, S. Sava, J. Rogowska, and D. A. Yurgelun-Todd, "A preliminary study of sex differences in brain activation during a spatial navigation task in healthy adults," *Perceptual and Motor Skills,* vol. 113, no. 2, pp. 461-480, 2011.
[251] T. Ohnishi, H. Matsuda, M. Hirakata, and Y. Ugawa, "Navigation ability dependent neural activation in the human brain: an fMRI study," *Neuroscience Research,* vol. 55, no. 4, pp. 361-369, 2006.
[252] R. J. Blanch, D. Brennan, B. Condon, C. Santosh, and D. Hadley, "Are there gender-specific neural substrates of route learning from different perspectives?," *Cerebral Cortex,* vol. 14, no. 11, pp. 1207-1213, 2004.
[253] B. Zimmerberg, H. L. Sukel, and J. D. Stekler, "Spatial learning of adult rats with fetal alcohol exposure: deficits are sex-dependent," *Behavioural Brain Research,* vol. 42, no. 1, pp. 49-56, 1991.
[254] P. A. May *et al.*, "Approaching the prevalence of the Full Spectrum of Fetal Alcohol Spectrum Disorders in a South African population-based study," *Alcoholism: Clinical and Experimental Research,* vol. 37, no. 5, pp. 818-830, 2013.
[255] E. Madge, A. Van den Berg, and M. Robinson, "Manual for the junior South African individual scales (JSAIS)," *Pretoria: HSRC,* 1981.
[256] A. M. Daugherty, P. Yuan, C. L. Dahle, A. R. Bender, Y. Yang, and N. Raz, "Path complexity in virtual water maze navigation: differential associations with age, sex, and regional brain volume," *Cerebral Cortex,* p. bhu107, 2014.

[257] I. Liu, R. M. Levy, J. J. Barton, and G. Iaria, "Age and gender differences in various topographical orientation strategies," *Brain Research,* vol. 1410, pp. 112-119, 2011.
[258] M. K. Rodgers, J. A. Sindone, and S. D. Moffat, "Effects of age on navigation strategy," *Neurobiology of Aging,* vol. 33, no. 1, pp. 202. e15-202. e22, 2012.
[259] N. J. Sandstrom, J. Kaufman, and S. A. Huettel, "Males and females use different distal cues in a virtual environment navigation task," *Cognitive Brain Research,* vol. 6, no. 4, pp. 351-360, 1998.
[260] D. M. Saucier, S. M. Green, J. Leason, A. MacFadden, S. Bell, and L. J. Elias, "Are sex differences in navigation caused by sexually dimorphic strategies or by differences in the ability to use the strategies?," *Behavioral Neuroscience,* vol. 116, no. 3, p. 403, 2002.
[261] S. D. Moffat, E. Hampson, and M. Hatzipantelis, "Navigation in a “virtual” maze: Sex differences and correlation with psychometric measures of spatial ability in humans," *Evolution and Human Behavior,* vol. 19, no. 2, pp. 73-87, 1998.
[262] E. Coluccia and G. Louse, "Gender differences in spatial orientation: A review," *Journal of Environmental Psychology,* vol. 24, no. 3, pp. 329-340, 2004.
[263] N. E. Andersen, L. Dahmani, K. Konishi, and V. D. Bohbot, "Eye tracking, strategies, and sex differences in virtual navigation," *Neurobiology of Learning and Memory,* vol. 97, no. 1, pp. 81-89, 2012.
[264] N. T. Nowak, A. Murali, and I. Driscoll, "Factors related to sex differences in navigating a computerized maze," *Journal of Environmental Psychology,* vol. 43, pp. 136-144, 2015.
[265] D. A. Hamilton *et al.*, "Effects of moderate prenatal ethanol exposure and age on social behavior, spatial response perseveration errors and motor behavior," *Behavioural Brain Research,* vol. 269, pp. 44-54, 2014.
[266] E. A. Maguire, N. Burgess, J. G. Donnett, R. S. Frackowiak, C. D. Frith, and J. O'Keefe, "Knowing where and getting there: a human navigation network," *Science,* vol. 280, no. 5365, pp. 921-924, 1998.
[267] G. Janzen and C. G. Weststeijn, "Neural representation of object location and route direction: an event-related fMRI study," *Brain Research,* vol. 1165, pp. 116-125, 2007.
[268] K. Jordan, J. Schadow, T. Wuestenberg, H.-J. Heinze, and L. Jäncke, "Different cortical activations for subjects using allocentric or egocentric strategies in a virtual navigation task," *Neuroreport,* vol. 15, no. 1, pp. 135-140, 2004.
[269] L. Frings *et al.*, "Precuneus is involved in allocentric spatial location encoding and recognition," *Experimental Brain Research,* vol. 173, no. 4, pp. 661-672, 2006.
[270] J. P. Aggleton, S. D. Vann, C. J. Oswald, and M. Good, "Identifying cortical inputs to the rat hippocampus that subserve allocentric spatial processes: a simple problem with a complex answer," *Hippocampus,* vol. 10, no. 4, pp. 466-474, 2000.
[271] S. D. Moffat, K. M. Kennedy, K. M. Rodrigue, and N. Raz, "Extrahippocampal contributions to age differences in human spatial navigation," *Cerebral Cortex,* vol. 17, no. 6, pp. 1274-1282, 2007.
[272] P. F. Rodriguez, "Human navigation that requires calculating heading vectors recruits parietal cortex in a virtual and visually sparse water maze task in fMRI," *Behavioral Neuroscience,* vol. 124, no. 4, p. 532, 2010.
[273] N. Dambeck *et al.*, "Interhemispheric imbalance during visuospatial attention investigated by unilateral and bilateral TMS over human parietal cortices," *Brain Research,* vol. 1072, no. 1, pp. 194-199, 2006.
[274] C. D. Chambers, J. M. Payne, M. G. Stokes, and J. B. Mattingley, "Fast and slow parietal pathways mediate spatial attention," *Nature Neuroscience,* vol. 7, no. 3, pp. 217-218, 2004.
[275] C. C. Hilgetag, H. Théoret, and A. Pascual-Leone, "Enhanced visual spatial attention ipsilateral to rTMS-induced'virtual lesions' of human parietal cortex," *Nature Neuroscience,* vol. 4, no. 9, pp. 953-957, 2001.
[276] J. Jonides, E. E. Smith, R. A. Koeppe, E. Awh, S. Minoshima, and M. A. Mintun, "Spatial working memory in humans as revealed by PET," *Nature,* vol. 363, no. 6430, pp. 623-625, 1993.
[277] J. M. Jansma, N. F. Ramsey, R. Coppola, and R. S. Kahn, "Specific versus nonspecific brain activity in a parametric N-back task," *Neuroimage,* vol. 12, no. 6, pp. 688-697, 2000.
[278] V. A. Diwadkar, P. A. Carpenter, and M. A. Just, "Collaborative activity between parietal and dorso-lateral prefrontal cortex in dynamic spatial working memory revealed by fMRI," *Neuroimage,* vol. 12, no. 1, pp. 85-99, 2000.
[279] L. Trojano *et al.*, "Matching two imagined clocks: the functional anatomy of spatial analysis in the absence of visual stimulation," *Cerebral Cortex,* vol. 10, no. 5, pp. 473-481, 2000.

[280] A. T. Sack, J. A. Camprodon, A. Pascual-Leone, and R. Goebel, "The dynamics of interhemispheric compensatory processes in mental imagery," *Science,* vol. 308, no. 5722, pp. 702-704, 2005.
[281] W. Richter *et al.*, "Motor area activity during mental rotation studied by time-resolved single-trial fMRI," *Journal of Cognitive Neuroscience,* vol. 12, no. 2, pp. 310-320, 2000.
[282] B. Alivisatos and M. Petrides, "Functional activation of the human brain during mental rotation," *Neuropsychologia,* vol. 35, no. 2, pp. 111-118, 1997.
[283] M. S. Cohen *et al.*, "Changes in cortical activity during mental rotation A mapping study using functional MRI," *Brain,* vol. 119, no. 1, pp. 89-100, 1996.
[284] M. Corbetta, "Frontoparietal cortical networks for directing attention and the eye to visual locations: Identical, independent, or overlapping neural systems?," *Proceedings of the National Academy of Sciences,* vol. 95, no. 3, pp. 831-838, 1998.
[285] C. R. Rosenthal, V. Walsh, S. K. Mannan, E. J. Anderson, M. B. Hawken, and C. Kennard, "Temporal dynamics of parietal cortex involvement in visual search," *Neuropsychologia,* vol. 44, no. 5, pp. 731-743, 2006.
[286] U. Leonards, S. Sunaert, P. Van Hecke, and G. A. Orban, "Attention Mechanisms in Visual Search—An fMRI Study," *Journal of Cognitive Neuroscience,* vol. 12, no. Supplement 2, pp. 61-75, 2000.
[287] R. Goebel, D. E. Linden, H. Lanfermann, F. E. Zanella, and W. Singer, "Functional imaging of mirror and inverse reading reveals separate coactivated networks for oculomotion and spatial transformations," *Neuroreport,* vol. 9, no. 4, pp. 713-719, 1998.
[288] P. A. Carpenter, M. A. Just, T. A. Keller, W. Eddy, and K. Thulborn, "Graded functional activation in the visuospatial system with the amount of task demand," *Journal of Cognitive Neuroscience,* vol. 11, no. 1, pp. 9-24, 1999.
[289] H. J. Spiers and E. A. Maguire, "A navigational guidance system in the human brain," *Hippocampus,* vol. 17, no. 8, pp. 618-626, 2007.
[290] S. D. Vann, J. P. Aggleton, and E. A. Maguire, "What does the retrosplenial cortex do?," *Nature Reviews Neuroscience,* vol. 10, no. 11, pp. 792-802, 2009.
[291] R. A. Epstein, "Parahippocampal and retrosplenial contributions to human spatial navigation," *Trends in Cognitive Sciences,* vol. 12, no. 10, pp. 388-396, 2008.
[292] B. A. Vogt, D. M. Finch, and C. R. Olson, "Functional heterogeneity in cingulate cortex: the anterior executive and posterior evaluative regions," *Cerebral Cortex,* vol. 2, no. 6, pp. 435-443, 1992.
[293] M. Arsalidou and M. J. Taylor, "Is 2+ 2= 4? Meta-analyses of brain areas needed for numbers and calculations," *Neuroimage,* vol. 54, no. 3, pp. 2382-2393, 2011.
[294] F. Galton, "Visualised numerals," *Nature,* vol. 21, no. 533, pp. 252-256, 1880.
[295] S. Dehaene, S. Bossini, and P. Giraux, "The mental representation of parity and number magnitude," *Journal of Experimental Psychology: General,* vol. 122, no. 3, p. 371, 1993.
[296] W. Fias, "The importance of magnitude information in numerical processing: Evidence from the SNARC effect," *Mathematical Cognition,* vol. 2, no. 1, pp. 95-110, 1996.
[297] S. Dehaene, E. Dupoux, and J. Mehler, "Is numerical comparison digital? Analogical and symbolic effects in two-digit number comparison," *Journal of Experimental Psychology: Human Perception and Performance,* vol. 16, no. 3, p. 626, 1990.
[298] W. Fias, "Two routes for the processing of verbal numbers: Evidence from the SNARC effect," *Psychological Research,* vol. 65, no. 4, pp. 250-259, 2001.
[299] X. Zhou, C. Chen, L. Chen, and Q. Dong, "Holistic or compositional representation of two-digit numbers? Evidence from the distance, magnitude, and SNARC effects in a number-matching task," *Cognition,* vol. 106, no. 3, pp. 1525-1536, 2008.
[300] J. Lammertyn, W. Fias, and J. Lauwereyns, "Semantic influences on feature-based attention due to overlap of neural circuits," *Cortex,* vol. 38, no. 5, pp. 878-882, 2002.
[301] M. D. de Hevia, L. Girelli, and G. Vallar, "Numbers and space: A cognitive illusion?," *Experimental Brain Research,* vol. 168, no. 1-2, pp. 254-264, 2006.
[302] H.-C. Nuerk, G. Wood, and K. Willmes, "The universal SNARC effect: The association between number magnitude and space is amodal," *Experimental Psychology,* vol. 52, no. 3, pp. 187-194, 2005.

[303] M. H. Fischer, A. D. Castel, M. D. Dodd, and J. Pratt, "Perceiving numbers causes spatial shifts of attention," *Nature Neuroscience,* vol. 6, no. 6, pp. 555-556, 2003.
[304] G. Galfano, E. Rusconi, and C. Umiltà, "Number magnitude orients attention, but not against one's will," *Psychonomic Bulletin & Review,* vol. 13, no. 5, pp. 869-874, 2006.
[305] M. Casarotti, M. Michielin, M. Zorzi, and C. Umiltà, "Temporal order judgment reveals how number magnitude affects visuospatial attention," *Cognition,* vol. 102, no. 1, pp. 101-117, 2007.
[306] Z. Cattaneo, J. Silvanto, A. Pascual-Leone, and L. Battelli, "The role of the angular gyrus in the modulation of visuospatial attention by the mental number line," *NeuroImage,* vol. 44, no. 2, pp. 563-568, 2009.
[307] J. C. Marshall and P. W. Halligan, "When right goes left: an investigation of line bisection in a case of visual neglect," *Cortex,* vol. 25, no. 3, pp. 503-515, 1989.
[308] M. Zorzi, K. Priftis, and C. Umiltà, "Brain damage: neglect disrupts the mental number line," *Nature,* vol. 417, no. 6885, pp. 138-139, 2002.
[309] P. Vuilleumier, S. Ortigue, and P. Brugger, "The number space and neglect," *Cortex,* vol. 40, no. 2, pp. 399-410, 2004.
[310] A. E. Cavanna and M. R. Trimble, "The precuneus: a review of its functional anatomy and behavioural correlates," *Brain,* vol. 129, no. 3, pp. 564-583, 2006.
[311] M. Piazza, A. Mechelli, B. Butterworth, and C. Price, "The quantifying brain: Functional neuroanatomy of numerosity estimation and counting," *Neuron,* vol. 44, pp. 547-555, 2002.
[312] L. Naccache and S. Dehaene, "The priming method: imaging unconscious repetition priming reveals an abstract representation of number in the parietal lobes," *Cerebral Cortex,* vol. 11, no. 10, pp. 966-974, 2001.
[313] M. J. Burden *et al.*, "The effects of fetal alcohol syndrome on response execution and inhibition: an event-related potential study," *Alcoholism: Clinical and Experimental Research,* vol. 33, no. 11, pp. 1994-2004, 2009.
[314] N. C. Dodge *et al.*, "Prenatal alcohol exposure and interhemispheric transfer of tactile information: Detroit and Cape Town findings," *Alcoholism: Clinical and Experimental Research,* vol. 33, no. 9, pp. 1628-1637, 2009.
[315] V. N. Kodali *et al.*, "Differential Recruitment of Brain Regions During Response Inhibition in Children Prenatally Exposed to Alcohol," *Alcoholism: Clinical and Experimental Research,* vol. 41, no. 2, pp. 334-344, 2017.
[316] M. M. Gerhold, S. W. Jacobson, J. L. Jacobson, C. D. Molteno, E. M. Meintjes, and C. M. Andrew, "An ERP study of response inhibition in the auditory domain in children with fetal alcohol spectrum disorders," *Alcoholism: Clinical and Experimental Research,* vol. 41, no. 1, pp. 96-106, 2017.
[317] B. R. Postle, M. D'Esposito, and S. Corkin, "Effects of verbal and nonverbal interference on spatial and object visual working memory," *Memory & Cognition,* vol. 33, no. 2, pp. 203-212, 2005.