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Hippocampal Abnormalities in Youth with Alcohol-Related Neurodevelopmental Disorder

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Abstract

Individuals diagnosed with alcohol-related neurodevelopmental disorder (ARND) exhibit difficulty on hippocampally mediated memory tasks and show reduced hippocampal size. However inconsistencies exist regarding the affected memory functions and where within the hippocampi effects occur. Given recent studies showing anterior and posterior segments support distinct memory functions and sex dimorphisms in hippocampal function, we asked whether these factors influence memory performance in youth with ARND ($n = 18$) and typically developing controls ($n = 17$). Participants received a battery of memory tests and a structural MRI scan. Right and left hippocampi were manually traced; anterior and posterior segments were delineated at the uncus. Measured were intracranial volumes (ICV) and right and left hippocampi and hippocampal segments. Volumes were adjusted for ICV. Relative to controls, the ARND group had lower IQs and memory performance on most tasks and marginally smaller ICVs. Left and right hippocampal volumes and posterior segments were smaller in the ARND group. Although no sex differences were observed between groups, females overall had larger anterior hippocampi than males. Positive and negative associations between hippocampal and selective memory indices were found in the ARND group only. These findings are the first to suggest that posterior hippocampal development may be compromised in youth with ARND. (*JINS*, 2014, *20*, 181–191)

Keywords: Fetal alcohol spectrum disorder (FASD), Sex differences, Hippocampus, Learning, Memory, Adolescence

INTRODUCTION

Approximately 2–5% of all children in North America and Europe have fetal alcohol spectrum disorder (FASD) (Chudley et al., 2005; May et al., 2009) and the incidence is much higher in South African black and Canadian First Nations populations (May et al., 2008; Robinson, Conry, & Conry, 1987). The severe deficits and behavioral problems associated with this condition (Kodituwakku, 2007) have made it a challenge for parents/caregivers, health professionals (Chudley et al., 2007), and the justice system alike (Burd et al., 2003; Fast & Conry, 2009). The range and severity of deficits in FASD are usually, although not always, reflective of the duration and amount of alcohol consumed, the gestational period of consumption (Autti-Rämö & Granstrom, 1991; Berman & Hannigan, 2000), and such mitigating factors as poverty, deprivation, pre- and postnatal undernutrition, multiple foster placements, and abuse and neglect (May & Gossage, 2011).

Within the fetal alcohol spectrum, the best-known condition is fetal alcohol syndrome (FAS), which is characterized by a constellation of features that include growth deficiency, distinct facial dysmorphia (e.g., palpebral fissures, long flat philtrum, thin vermillion), and significant central nervous system (CNS) impairments (Stratton, Howe, & Battaglia, 1996). However the most prevalent form of FASD, estimated at 10 times the frequency of FAS (Stoler & Holmes, 1999), is alcohol-related neurodevelopmental disorder (ARND), which involves only CNS abnormalities (Clarren & Smith, 1978; Riley & McGee, 2005; Stratton et al., 1996). Because children with ARND lack defining physical features for clinical recognition, a substantial proportion may be misdiagnosed or wrongly diagnosed (Chudley et al., 2007), signifying an even higher prevalence than formerly estimated.

Research on persons with FASD has revealed IQ reductions (Rasmussen et al., 2008), cognitive impairments (Astley, Carmichael Olson, et al., 2009; Nash et al., 2013), and social and behavioral difficulties (Stevens et al., 2013;

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see Donaldson et al., 2011) as well as predisposition to mental health issues (Pei, Rinaldi, Rasmussen, Massey, & Massey, 2008). These include attention deficit disorder (Nash et al., 2006; O'Malley & Nanson, 2002; Rasmussen et al., 2010), conduct disorder (Nash, Koren, & Rovet, 2011), and autism (Bishop, Gahagan, & Lord, 2007; Stevens, Nash, Koren, & Rovet, 2012). As adults, individuals with FASD typically do not complete their educations and are unemployed while many experience depression, suicide risk, and trouble with the law (O'Malley & Huggins, 2005; Streissguth et al., 2004). Consequently, FASD confers an enormous life-long burden and a high cost to society (Lupton, Burd, & Harwood, 2004; Stade, Ungar, Stevens, Beyene & Koren, 2006; Stade et al., 2009).

In recent years, extensive literature has emerged on the neuroanatomic characteristics of FASD. Findings indicate global brain volume reductions (Archibald et al., 2001) with reduced size of specific brain regions including parietal, temporal, and frontal lobes (Lebel, Rousette, & Sowell, 2011; Sowell et al., 2002; Spadoni, McGee, Fryer, & Riley, 2007), caudate (Cortese et al., 2006), cerebellum (Sowell et al., 1996), corpus callosum (Autti-Rämö et al., 2002; Riley et al., 1995), and hippocampus (Coles et al., 2011; Willoughby, Sheard, Nash, & Rovet, 2008). Also observed are cortical and subcortical gray matter reductions (Astley, Aylward, et al., 2009; Nardelli, Lebel, Rasmussen, Andrew, & Beaulieu, 2011), abnormalities in cortical morphology (Sowell et al., 2008; Yang et al., 2012; Zhou et al., 2011), white matter irregularities (Lebel et al., 2008; Wozniak et al., 2009), and functional disturbances (Fryer, McGee, Matt, Riley, & Mattson, 2007; Malisza et al., 2005; Sowell et al., 2007). Studies examining children with ARND almost exclusively (Rajaprakash, Chakravarty, Lerch, & Rovet, 2014; Willoughby, Sheard, Nash, & Rovet, 2008) or *versus* other FASD forms (Astley, Aylward et al., 2009; Lebel et al., 2008) have found similar neuroanatomic effects between subgroups as in the entire FASD group.

Among individuals with FASD, one consistent finding is a weakness in memory skills (Mattson, Riley, Elis, Stern, & Lyons, 1996; Rasmussen, Horne, & Witol, 2008; Richardson, Ryan, Willford, Day, & Goldschmidt, 2002; Uecker & Nadel, 1996), particularly on hippocampally mediated tasks (Willford, Richardson, Leech, & Day, 2004). In a study using a virtual Morris Water Maze task, the prototype for studying the hippocampus in rodents, individuals with heavy prenatal alcohol exposure (PAE) exhibited comparable learning and memory deficits as the hippocampally damaged rodents (Hamilton, Kodituwakku, Sutherland, & Savage, 2003). Although studies specifically of the ARND subgroup have reported either equivalent (Astley, Carmichael Olson, et al., 2009) or milder deficits as in FAS (Coles et al., 2011), inconsistencies exist among studies. For example, Willoughby et al. (2008) reported youth with ARND had a wide range of verbal and nonverbal memory weaknesses relative to controls, whereas Rasmussen, McCauley, & Andrew (2008) comparing those with an ARND diagnosis and those with PAE not meeting diagnostic criteria for FASD found the only differences were in face recognition and number repetition and groups otherwise performed similarly. Thus the specific memory difficulties of children with ARND are not yet known.

When the hippocampus was directly examined in individuals with FASD, structural (e.g., Autti-Rämö et al., 2002) and functional (Sowell et al., 2007) abnormalities, as well as differences, in laterality were observed (Riikonen, Salonen, Partanen, & Verho, 1999). However in the ARND subgroup specifically, studies showed varying findings. For example, Astley, Aylward, et al. (2009) observed both ARND and FAS youth aged 8 to 16 years had significantly smaller right and left hippocampi than controls. In contrast, Willoughby et al. (2008) studying ARND youth primarily observed only left hippocampal volume reductions, whereas Coles et al. (2011) studying adults with heavy PAE found memory deficits were mediated by right hippocampal volume reductions but only if they showed facial dysmorphia (i.e., FAS but not ARND).

In hippocampal research, a recent emphasis has been the ascription of different memory functions to specific hippocampal subregions (Moser & Moser, 1998). According to Poppenk and Moscovitch (2011), the anterior hippocampus subserves the encoding of new information (Fanselow & Dong, 2010), whereas the posterior hippocampus contributes to recollective aspects of memory and event reconstruction (see also, Poppenk, Evensmoen, Moscovitch, & Nadel, 2013). For example, London taxi drivers, who have a vast knowledge of spatial locations, show enlarged posterior hippocampi at the expense of their anterior hippocampus (Maguire et al., 2000). Poppenk and Moscovitch (2011) studying young adults observed those with large posterior but small anterior hippocampal regions had better recall of previously learned proverbs than age-matched counterparts. However, it is not known (a) whether posterior and anterior regions are differentially affected in children with FASD, and the ARND subgroup specifically, and (b) what are the implications of regional differences for specific memory difficulties.

An extensive literature on rodents with prenatal ethanol exposure has shown severe memory impairments and associated hippocampal abnormalities (Berman & Hannigan, 2000; Klintsova et al., 2007; Livy, Miller, Maier, & West, 2003). While mice exposed to ethanol at gestational day 7 exhibited the FAS facial deformity, those exposed later had different or no unusual facial features, as in ARND (Lipinski et al., 2012), however, they still showed hippocampal volume reductions, especially on the right side (Parnell et al., 2009). Additionally, ethanol-exposed rodents show sex differences in hippocampally mediated memory functions. For example, exposed females had larger encoding deficits than males (Minetti, Arolfo, Virgolini, Brioni, & Fulginiti, 1996), who instead showed larger recall deficits than females (Kelly, Leggett, & Cronise, 2009). Furthermore, when rodents were exposed to ethanol in the period corresponding to the human third trimester, males and females both had spatial learning impairments but males exhibited deficits with just 2 days of exposure while the females needed 4 days, suggesting greater male vulnerability (Goodlett & Peterson, 1995).

In humans *not* exposed gestationally to alcohol, sexual dimorphisms in hippocampal volume are consistently reported. Females, for example, typically show larger hippocampi (per brain size) and more rapid rates of hippocampal growth than males (Filipek, Richelme, Kennedy, & Caviness, 1994; Giedd et al., 1996; Giedd, Castellanos, Rajapakse, Vaituzis, & Rapoport, 1997), a finding attributed to hormonal effects (Lord, Buss, Lupien, & Preussner, 2008). Nevertheless inconsistencies exist as to what hippocampus is most affected in males *versus* females and where within the hippocampus disturbances occur. Neufang et al. (2009) found that in 8- to 15-year-olds, females had larger hippocampi bilaterally than same-age males. In contrast, Gogtay et al. (2006) found in typically developing youth aged 4 to 18 years, sex differences were confined to specific hippocampal subregions. These reflected more prominent growth in the left posterior hippocampus and greater volume loss at the posterior hippocampal pole in females than males and the greater age-related volume loss at the head of the hippocampus in males. However, sex differences in hippocampal volumes have not been studied in youth with FASD, particularly those with the ARND subtype.

The present study was conducted in the context of a larger investigation of memory and the hippocampus in children with the ARND variant of FASD. Currently, we sought to address some of the knowledge gaps and inconsistencies identified above. Specifically, we asked whether youth with ARND differ from non-exposed typically developing controls in memory abilities, size of their hippocampi and specific hippocampal segments, sex differences in memory functions and hippocampal structure, and correlations between specific memory deficits and hippocampal reductions.

METHODS

Participants

Participants were 35 children and adolescents aged 11.1 to 14.8 years, 18 with a diagnosis of ARND along the FASD spectrum and 17 typically developing controls. All had no MRI counter-indications (e.g., braces, implants).

The ARND group consisted of 11 males and 7 females previously diagnosed at the Motherisk Follow-up Clinic at the Hospital for Sick Children (SickKids), a regional FASD diagnostic facility, which included a pediatrician, several psychologists and psychometrists, and a speech therapist. Most children attending this clinic were brought by a foster or adoptive parent who sought to ascertain whether the child's current cognitive or behavioral problems were related to PAE and then obtain the necessary services. To be assessed in this clinic, a history of PAE first had to be confirmed from direct report of the mother or a relative or valid documentation that the mother was an alcoholic or received treatment for alcoholism during pregnancy or had the child removed by the Children's Aid Society (CAS) at birth due to her alcohol abuse. If suitable, the child received a thorough physical and neurological assessment by the pediatrician and a detailed medical history was obtained from the caregiver or an also accompanying social worker. Finally, children underwent a comprehensive neuropsychological evaluation. Children diagnosed with FAS showed the requisite facial dysmorphology, growth retardation, and either an IQ score below 70 or a neuropsychological profile indicating deficits (scores 2 standard deviations below test mean) on subtests in any three of the domains specified by the Canadian FASD Diagnostic Guideline system (Chudley et al., 2005). Children not showing the physical features but having the requisite neuropsychological profile were considered to have ARND. Because the partial FAS classification was not being used in the clinic at the time current participants received their diagnoses, all children were classified as having ARND. Only children with IQs >70 were included presently.

Controls were 17 (10 males and 7 females) typically developing children recruited from among non-ARND foster or step-siblings, participant lists from previous studies, and local advertising within SickKids. They were matched for age (within 6 months) and sex with children in the ARND group. All of their mothers reported not drinking alcohol or taking medications for a major illness during pregnancy. Any child with a reported neurological disease, head injury, chronic illness, learning disability, psychiatric disorder, or obtaining a low IQ score was excluded.

PROCEDURES

The study consisted of two visits over a 6-month period. The first session involved a 4-hr assessment that included an intelligence test, multiple tests of memory and other abilities (e.g., executive functioning, reported elsewhere). The assessment was conducted by a team of psychometrists and advanced graduate students trained on all tasks and masked as best as possible to group status. Snack and lunch breaks were provided as needed. The second session involved two 1-hr same-day MRI scans in a 1.5 Tesla Signa Excite (General Electric, Milwaukee, WI) scanner in the SickKids Diagnostic Imaging Unit. Both structural and functional sequences were performed (functional results described elsewhere; e.g., Rovet, Sheard, Wheeler, & Skocic, 2010). To minimize movement during structural scanning, children viewed movies *via* MRI-compatible goggles.

Initially, parents/caregivers gave written consent and participants gave oral assent. After each session, participants received two movie passes and a certificate of participation for high school credit hours; parents/caregivers were compensated for travel expenses. At the end of scanning, participants also received a CD containing their own brain images. Parents/caregivers received a detailed report of the child's performance within 2 months of the assessment. Additionally, all scans were reviewed for gross abnormalities by a staff neuroradiologist masked to group status and her report was sent to each child's physician. The Research Ethics Board at SickKids approved all procedures.

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Tests and Measures

The following tests were currently selected from our larger test battery: Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) based on Vocabulary and Matrix Reasoning subtests; selective Children's Memory Scale subtests (CMS; Cohen, 1997); complete Test of Memory and Learning (TOMAL; Reynolds & Bigler, 2007); Rey-Osterrieth Complex Figure task (ROCF; Bernstein & Waber, 1999; Osterrieth & Rey, 1944; Taylor, 1991); and three Cambridge Neuropsychological Test Automated Battery (CANTAB, 1998) subtests (see Table 1).

Image Acquisition and Processing

The structural sequence consisted of a 7-min high-resolution axial T1 3D FSPGR sequence (fast spoiled gradient recalled echo) with inversion recovery to provide increased T1 weighting and allow for enhanced contrast of gray and white matter tissue. Approximately 125 1.5-mm-thick slices were obtained per scan to provide whole brain coverage. T1 image acquisition parameters were: repetition time = 10.3 ms, echo time = 4.2 ms, inversion time = 400 ms, flip angle = 20°, and a 256×192 acquisition matrix. Three additional clinical sequences (10-min) read by the neuroradiologist for gross brain abnormalities were: a Sagittal T1 Flair, a Coronal T2 Fast Relaxation Fast Spin Echo, and an Axial T2 Flair.

All images were subsequently transferred to a Linux workstation running Fedora 7 (Red Hat, Raleigh, NC). In Analyze 9.0 (Mayo Clinic, Rochester, MN), Dicom files were reconstructed to have isotropic voxels of less than 1 mm and transformed into standard space using AC-PC (anterior commissure-posterior commissure) alignment. For all tracing, an optical wheel mouse was used to manually define regions of interest (ROIs). Right and left hippocampi were identified in each plane and verified using pre-determined landmarks (Duvernoy, 2005; Pruessner et al., 2000; Willoughby et al., 2008). Each hippocampus included volumes of the dentate gyrus, subicular complex, and the cornu ammonis regions, but not the fimbria or alveus, which served as boundaries; the entorhinal cortex was not measured. This conservative tracing approach was adopted to minimize partial voluming effects.

Hippocampi were traced in the coronal plane in an anterior-to-posterior direction beginning at the rostral end

Table 1. Descriptions of cognitive measures

Test	Subtest	Description	Domain
CMS	Dots	Learn locations of dot markers in a matrix for immediate and delayed recall	Visual learning/ recall
	Stories	Listen to and repeat a story immediately and after a delay	Verbal memory
	Picture Locations	View a series of matrices containing an increasing number of objects; point to locations of objects in empty matrix	Visual item recognition
	Faces	Learn and remember a series of faces immediately and after a delay	Visual item recognition
TOMAL	Word Selective reminding	Learn 12 words over 8 trials; recall immediately and after delay	Verbal learning/ recall
	Visual Selective reminding	Remember and point to dots on a page immediately and after a delay	Verbal learning/ recall
	Object recall	See a series of pictures and recall their names verbally	Verbal item recognition
	Digits	Repeat sequence of digits in forward and backward order	Memory span
	Facial memory	See a face and then recognize it in among a series of distracters immediately and after a delay	Visual item recognition
	Abstract visual memory	See an abstract figure and then find it in a series of distracters	Visual item recognition
	Sequential memory	Look at a series of geometric designs and recall the order in which they were presented	Memory for sequences
ROCF	Copy	Copy complex figure drawings and reproduce immediately without model and after a delay	Visual learning/ recall
CANTAB	Paired Associates Learning (PAL)	Remember the location of patterns in 1 to 6 boxes that appear on the screen.	Working memory
	Spatial Span (SSP)	View white squares that randomly change color. Touch boxes that changed color in same order as displayed	Sequence memory
	Spatial Working Memory (SWM)	View a set of colored boxes, only one of which contains a token. Find target by pointing to boxes without touching a box twice	Working memory

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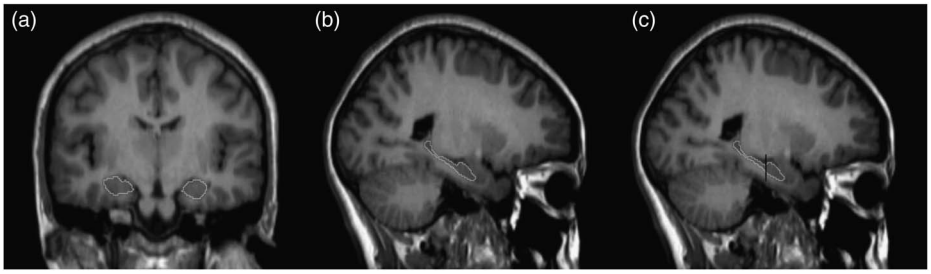

Fig. 1. a: Coronal view of hippocampus. b: Right sagittal view of hippocampus; c: Right sagittal view of anterior and posterior hippocampal regions.

when the head first appeared below the amygdala and terminating at the caudal end when the crura of the fornices separate from the hippocampal tail (Figure 1a). ROIs were further examined in sagittal (Figure 1b) and axial planes to verify superior and inferior boundaries. The anterior region was defined by the emergence of the uncal recess of the hippocampal head in the superomedial region of the hippocampus; the posterior division was defined as the first appearance of ovoid mass of gray matter inferomedial to the trigone of the lateral ventricle (Figure 1c; see also Poppenk et al., 2013). The first author (J.D.) traced all hippocampi and delineated their anterior and posterior regions while the second author (J.S.) traced ~25% of scans. Their inter-rater reliabilities using Cronbach's alpha were 0.91 and 0.84 for left and right hippocampal volumes, respectively. Intracranial volume (ICV), consisting of total gray matter, white matter, and cerebrospinal fluid (CSF), was determined using Christian Gaser's VBM Toolbox v.1.18 for SPM5.

Statistical Analyses

Analyses were conducted in SPSS version 21.0 for Macintosh. Regarding demographic indices, categorical variables were analyzed for group differences using χ^2 and continuous variables (viz., age, intelligence, ICV, and total hippocampal volume) using *t* tests. Effects of handedness on hippocampal volumes were determined using the Mann-Whitney *U* test. To assess effects on memory, we conducted separate group by sex multivariate analyses of variance (MANOVA) for each global test and examined effects on individual subtests *via* the univariate analyses provided within each MANOVA. Hippocampal measurements were examined for group and sex differences by two methods: (i) repeated measures analysis of variance (ANOVA) with group and sex as between-group factor variables and side as the repeated measure and (ii) group by sex MANOVA on the four individual segments. For all analyses, effect sizes were determined using partial eta-squared. To ascertain the relations between memory and hippocampal size, we performed partial correlations with age removed. To restrict the number of correlations and possibility of a type-1 statistical error, we used only memory indices showing highly significant ($p < .005$) between-group differences. For all analyses, significance was set at $p < .05$.

RESULTS

Demographic Measures

Table 2 which presents the demographic data, shows that groups did not differ in age, sex, or handedness. However they did differ in their caregiving environments ($p < .001$), secondary prenatal drug exposures ($p < .001$), receipt of medications ($p < .001$), and comorbid ADHD diagnoses ($p < .001$). Specifically, the ARND group was more likely than controls to be (a) living in foster or adoptive homes rather than with biological mothers; (b) prenatally exposed to cigarettes, cocaine, or marijuana; (c) currently receiving stimulant medications; and (d) diagnosed with ADHD. Groups also differed in Full Scale IQ [$F(1,31) = 43.4$; $p < .001$] with the ARND group scoring below controls. However, we chose not to use IQ as a covariate in light of the Dennis et al. (2009) claim that this is unnecessary when IQ is a defining feature of a neurodevelopmental disability. The ARND group also scored below controls on WASI Vocabulary [$F(1,31) = 31.7$; $p < .001$] and Matrix Reasoning subtests [$F(1,31) = 73.2$; $p < .001$].

Memory Test Results

Table 3 presents both groups' mean scores on the subtests from the various memory tests. MANOVAs performed separately for each instrument revealed significant omnibus group differences on all instruments: CMS [$F(10,22) = 3.095$; $p = .013$; $\eta^2 = .585$], TOMAL [$F(12,20) = 3.184$; $p = .011$; $\eta^2 = .656$], CANTAB [$F(3,29) = 6.725$; $p = .001$; $\eta^2 = .410$], and Rey-O [$F(2,32) = 3.61$; $p = .028$; $\eta^2 = .184$]. There were no sex differences or group by sex interactions. Univariate analyses contained within each MANOVA showed the ARND group scored significantly below controls on: (i) every CMS subtest, except Dots Learning (effect sizes ranged from .168 for Dots Long Delay to .330 for Dots Short Delay); (ii) every TOMAL subtest except Word and Visual Delayed Selective Reminding (effect sizes ranged from .126 for Visual Selective Reminding to .410 for Visual Sequential Recall); (iii) all CANTAB subtests (effect sizes ranged from .168 for Spatial Span to .241 for Paired Associates Learning); and (iv) both Rey-O subtests ($\eta^2 = .157$ and .125 for Copy and Delayed Recall, respectively). These results suggest a broad spectrum of memory deficiencies in children with ARND.

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Table 2. Demographic, IQ, and brain volume information for ARND and Control groups

	ARND ($n = 18$)	Control ($n = 17$)
Mean (SD) age in years	12.9 (1.3)	12.3 (1.3)
Sex (% male)	61.1	62.4
Handedness (% right-handed)[a]	88.9	75.8
Right	16	12
Left	1	2
Unable to determine	1	3
Home care (% of cases)[b]		
With biological mother or relative	16.7	100
In foster care	22.2	0
With adoptive parents	50.0	0
Secondary prenatal exposures (%)		
Cigarettes	50.0	5.9
Cocaine	22.2	0
Marijuana	1	0
Comorbidity (% ADHD)	61.6	0
Current medications[c]		
Ritalin	16.7	0
Concerta	22.2	0
Dexadrine	5.6	0
Strattera	16.7	0
Citalopram	11.1	0
Intelligence[d]		
Full Scale IQ	91.6 (9.5)	112.9 (9.4)
Vocabulary (scaled score)	8.1 (2.4)	12.4 (2.4)
Matrix Reasoning (scaled score)	9.0 (2.6)	12.0 (1.7)
Intracranial volumes (cm^3)	1592.2 (288)	1745.1 (130)

[a]Handedness information was missing on 1 ARND and 3 controls; [b]Home care information was missing on 2 ARND; [c]All for ADHD; [d]Presented as Mean (SD)

ICV and Hippocampal Measurements

As preliminary analyses revealed no effects of handedness or age (range of r-values: −0.058 to −0.128) on hippocampal volumes, we combined left and right-handed participants and did not use age as a covariate in subsequent analyses. However, as we found a marginally significant effect of overall brain size with the Mann-Whitney U test applied ($z = -1.72$, $p = 0.086$, See Table 2), we used proportion scores adjusting hippocampal values for ICV in subsequent analyses. Table 4 contains the groups' mean raw hippocampal measurements while Figure 2 shows the proportion scores.

To examine for group and sex differences on hippocampal proportions, we first conducted a repeated measures ANOVA with group and sex as between-subjects factors and side as the repeated measure. Results revealed significant main effects of group [$F(1, 31) = 12.03$; $p = 0.002$, $\eta^2 = .28$] and sex [$F(1, 31) = 10.31$; $p = 0.003$, $\eta^2 = .25$] but no effect of side or any interactions. Both hippocampi were smaller in the ARND group than controls.

The next analysis involved a group by sex MANOVA on the four segments. Results revealed significant omnibus effects for group [$F(4,28) = 3.36$; $p = .023$; $\eta^2 = .325$] and sex [$F(4,28) = 3.53$; $p = .019$; $\eta^2 = .335$] and no group by sex interaction. Univariate analyses (provided within MANOVA) indicated the ARND group had smaller right and left posterior segments [$p = .001$ and .004, respectively] but not anterior hippocampal segments than controls, as shown in Figure 2. In contrast, the significant sex difference reflected the smaller anterior [p-values: right = .001; left = .003] but not posterior volumes in males than females.

Structure-Function Correlations

Although groups did not differ in age, we chose to partial out the effects of age from the subsequent correlational analyses given previous findings of different age trajectories for structure-function correlations with the hippocampus (Giedd et al., 1996). To limit the possibility of a Type-1 statistical error, we used only memory indices showing a highly significant group difference ($p < .005$; see Table 3). As there were no lateralized group differences, we combined results across right and left hippocampi or segments in these analyses.

For the ARND group, results revealed significant positive correlations between: (i) CMS Dots Short Delay and total hippocampal size ($r = 0.440$; $p = .007$), (ii) CMS Stories Immediate Recall and overall and anterior hippocampal volumes ($r = 0.664$, $p = .004$ and $r = 0.537$, $p = .026$), and (iii) CMS Stories Delayed Recall and global and anterior hippocampal volumes ($r = 0.707$; $p = .001$ and $r = 0.616$; $p = .009$). However, a significant negative correlation was also observed between TOMAL Visual Sequential Recall and total hippocampal volume ($r = -0.560$; $p = .019$). No significant correlations were observed for controls.

DISCUSSION

The present study sought to examine whether youth with ARND show memory weaknesses and reduced hippocampal size. Our findings revealed that relative to controls, youth with ARND exhibited a broad spectrum of memory difficulties, a marginally reduced brain size, and smaller right and left hippocampi, particularly in posterior subregions. Unlike rodents with prenatal ethanol exposure, however, we did not observe any sexual dimorphisms between ARND and control groups. Instead, females in both groups had larger hippocampi (adjusted for brain size) than did males, especially in their anterior hippocampal segments. ARND and control groups also differed in their patterns of structure/function correlations with better story recall being associated with larger global and anterior hippocampal volumes and better visual sequential recall being associated with smaller global hippocampal volumes, in the ARND group only.

Although current findings showed the ARND group was significantly outperformed by controls on most memory indices, it might be argued that since our ARND group was cognitively impaired, this group by definition would have had memory problems. However, it should be noted that to be assigned an ARND diagnosis using the Canadian Guidelines

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Table 3. Mean (SD) Memory Test Results for ARND and Control groups

Test	ARND *M (SD)*	Control *M (SD)*	Effect size*	
			p	(η^2)
Children's Memory Scale				
Dots: Learning	10.0 (2.8)	11.4 (2.6)	0.131	.068
Dots: Short Delay recall	9.0 (3.2)	12.3 (1.1)	0.000	.330
Dots: Long Delay recall	10.2 (2.9)	12.2 (1.3)	0.014	.168
Dots: Total recall	9.5 (2.7)	12.0 (2.0)	0.004	.231
Stories: Immediate recall	9.3 (3.2)	12.6 (2.4)	0.001	.268
Stories: Delayed recall	8.2 (1.9)	12.5 (2.8)	0.001	.275
Picture Locations: Immediate recall	9.6 (2.8)	11.8 (3.3)	0.034	.129
Picture Locations: Delayed recall	9.7 (2.6)	11.8 (3.3)	0.043	.118
Faces: Immediate recall	6.1 (3.3)	9.4 (4.1)	0.012	.175
Faces: Delayed recall	5.4 (3.8)	9.3 (4.6)	0.010	.185
Test of Memory and Learning				
Word Selective Reminding	10.7 (3.1)	12.9 (2.1)	0.017	.162
Word Selective Reminding Delayed	9.7 (2.1)	10.7 (1.4)	0.14	.074
Visual Selective Reminding	8.3 (3.1)	10.3 (3.2)	0.036	.126
Visual Selective Reminding Delayed	9.3 (1.9)	10.3 (1.2)	0.19	.091
Object Recall	7.7 (3.5)	11.0 (3.4)	0.008	.197
Digits Forward	5.4 (2.5)	8.2 (2.9)	0.004	.224
Digits Backward	8.4 (2.5)	10.9 (3.2)	0.015	.166
Facial Memory: Immediate	7.9 (3.0)	11.0 (3.0)	0.004	.221
Facial Memory: Delayed	8.9 (1.6)	10.6 (1.6)	0.003	.234
Abstract Visual Memory	9.8 (2.1)	12.4 (2.6)	0.002	.246
Visual Sequential Memory	7.6 (2.6)	12.1 (3.1)	0.000	.401
Rey-Osterrieth Complex Figures				
Copy	−2.0 (1.7)	−.74 (1.25)	0.019	.157
Delayed recall	−1.67 (1.20)	−.84 (1.02)	0.037	.125
Cambridge Neuropsychological Test Automated Battery				
Paired Associates Learning (PAL)	−.09 (.73)	.58 (.41)	0.002	.248
Spatial Span (SS)	−.49 (1.0)	.42 (1.1)	0.015	.168
Spatial Working Memory (SWM)	−.98 (.76)	−.23 (.86)	0.010	.186

*Effect size reflects partial eta-squared.

(Chudley et al., 2005), one needs to show impairments in any three of the multiple domains listed and memory deficits alone are not sufficient for diagnosis. Relevantly, Nash et al. (2013) reported considerable heterogeneity in the neuropsychological profile of children receiving an ARND diagnosis from our clinic while few areas of memory were affected in the clinic sample as a whole.

Table 4. Mean (SD) hippocampal volume (mm^3) measurements for ARND and Control groups

	ARND ($n = 18$)	Control ($n = 17$)
RH Anterior	636.7 (123.9)	787.6 (105.1)
RH Posterior	632.1 (103.3)	929.4 (176.2)
Total Right	1268.8 (119.6)	1716.5 (218.1)
LH Anterior	584.7 (103.4)	729.5 (90.9)
LH Posterior	660.9 (102.4)	924.5 (163.7)
Total Left	1245.6 (124.0)	1654.6 (188.8)
Total Left & Right	2514.4 (196.2)	3371.1 (373.1)

Group differences in memory varied between tasks as well as across subtests for each instrument. Largest differences were observed when participants had to remember a visual sequence and recall story details after a delay or a dot pattern immediately. Groups did not differ on word and object selective reminding tasks of the TOMAL or learning a dot pattern on the CMS, which are thought to engage the hippocampus (Zimmerman et al., 2008). Selective reminding results are inconsistent with findings from adults with PAE showing correlations between performance and right hippocampal size (Coles et al., 2011). However, it should be noted that in the latter study, persons with the most facial dysmorphia (i.e., FAS) showed effects and this was not present in our sample. According to Brickman, Stern, and Small (2011), selective reminding engages the entorhinal cortex primarily, which we did not include in our conservative tracing approach. Therefore, the spared performance of our ARND

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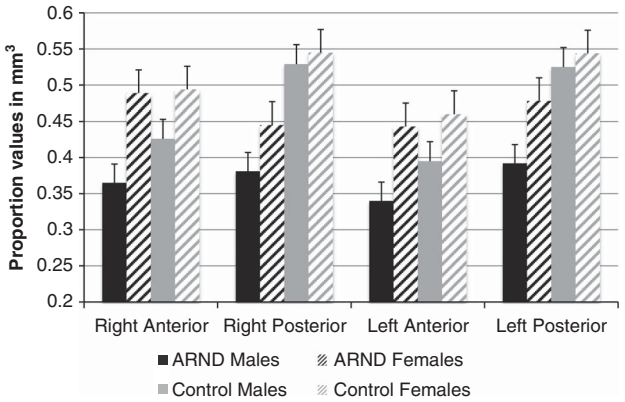

Fig. 2. Intracranial volumes (ICV) -adjusted hippocampal volumes (with standard error bars) showing right and left anterior and posterior regions for alcohol-related neurodevelopmental disorder (ARND) males (dark bars) and females (dark striped bars) and control males (light bars) and females (light striped bars). Univariate analyses within multivariate analyses of variance indicated the ARND group differed significantly from controls in right ($p = .001$) and left ($p = .004$) posterior segments (smaller in ARND), whereas males had significantly smaller right ($p = .001$) and left ($p = .003$) anterior hippocampal segments than females.

group may reflect normal development of this area in youth with ARND. Regarding the Dots task, it is interesting to note that while the ARND group performed adequately in the learning (i.e., encoding) phase, they did show difficulty reconstructing it subsequently.

Current findings showed that youth with ARND had smaller hippocampi bilaterally than did controls, especially in the posterior segment. In contrast, males from both groups showed smaller anterior hippocampal segments than did females. The latter finding is consistent with previous research showing larger bilateral hippocampi in females than males (Neufang et al., 2009) and that age-related volume loss is greater in posterior hippocampal regions in males than females (Gogtay et al., 2006). Although our sample's lack of a sexual dimorphism in hippocampal size is inconsistent with research on ethanol-exposed rodents, other aspects of hippocampal structure and function that may be sexually dimorphic in humans with PAE still need to be examined. Notably, the smaller anterior hippocampal region in males did not contribute to any sex differences in memory performance.

According to Poppenk et al. (2013), the posterior portion of the long axis of the hippocampus, which includes the dentate gyrus, is critical for recall and event reconstruction, whereas the anterior portion includes substructures needed for encoding the event. Our findings from the CMS Dots task are consistent with this notion since we observed groups differed in recalling the pattern but not in learning it initially and they also differed in posterior hippocampal size. In the present study, both right and left hippocampi were similarly affected in the ARND group supporting observations of Astley, Aylward, et al. (2009) on a slightly younger ARND group, but not our own previous findings of a left hippocampal effect (Willoughby et al., 2008) or that of Coles et al. (2011) showing a right hippocampal effect in adults with PAE. Differences from our earlier study may be explained by several factors: the slightly younger age distribution of our current sample, different distributions of FASD subtypes with none currently having FAS, and our current more conservative tracing approach.

Different structure-function relation patterns were observed between groups with hippocampal size being correlated with selective memory indices only in the ARND group. Our findings of a positive association between story recall with global and anterior hippocampal volumes, and a negative association between visual sequential recall and global hippocampal volumes in the ARND group only, may mean controls used other regions to efficiently recall the story while the ARND group relied heavily on their hippocampi. As we did not study other brain regions currently, we cannot address this issue.

Strengths of our study include: (i) examining children with ARND exclusively, (ii) studying children within a relatively narrow age range, and (iii) subdividing hippocampi into anterior and posterior segments, which is novel within the FASD population. However, several study limitations warrant further discussion. Our sample size was quite small, thus precluding our ability to find meaningful sex dimorphisms or structure-function correlations. Additionally, we were not able to control for mediating factors such as medication usage, comorbidity, family adversity, poor nutrition, stress, any of which could have affected the memory or hippocampal results. However supplementary analyses comparing hippocampal volumes by these factors (e.g., secondary prenatal drug exposures and ADHD comorbidity) failed to show any meaningful effects on hippocampal size (data not shown). Also our conservative tracing approach did not allow us to examine for regions such as the fimbria or parts of subiculum and well as cortical regions such as the entorhinal cortex, which may have been important for some of the memory indices on which we did obtain effects. Finally, current results were based solely on volumes and so did not examine other aspects of hippocampal integrity (viz., shape, contour), which may be sensitive to effects of PAE or to sex differences. Thus, future hippocampal studies using higher resolution MRI to allow for these finer analyses are warranted.

Overall, current findings showed reduced posterior hippocampal volumes and selective memory deficits in youth with ARND. These findings have implications for treating the memory difficulties of children with ARND that can impact on their school functioning, everyday memory functions (Agnihotri, Sheard, Keightley, & Rovet, 2012), and social relationships. Since techniques to facilitate recall and maintain newly learned events in memory, as well as to improve hippocampal integrity (e.g., exercise, music training, memory games), would also be beneficial for this population, future studies need to address these possibilities.

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