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COGNITIVE NEUROSCIENCE

Oculomotor control in children with fetal alcohol spectrum disorders assessed using a mobile eye-tracking laboratory

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Abstract

Prenatal exposure to alcohol can result in a spectrum of adverse developmental outcomes, collectively termed fetal alcohol spectrum disorders (FASDs). This study evaluated deficits in sensory, motor and cognitive processing in children with FASD that can be identified using eye movement testing. Our study group was composed of 89 children aged 8–15 years with a diagnosis within the FASD spectrum [i.e. fetal alcohol syndrome (FAS), partial fetal alcohol syndrome (pFAS), and alcohol-related neurodevelopmental disorder (ARND)], and 92 controls. Subjects looked either towards (prosaccade) or away from (antisaccade) a peripheral target that appeared on a computer monitor, and eye movements were recorded with a mobile, video-based eye tracker. We hypothesized that: (i) differences in the magnitude of deficits in eye movement control exist across the three diagnostic subgroups; and (ii) children with FASD display a developmental delay in oculomotor control. Children with FASD had increased saccadic reaction times (SRTs), increased intra-subject variability in SRTs, and increased direction errors in both the prosaccade and antisaccade tasks. Although development was associated with improvements across tasks, children with FASD failed to achieve age-matched control levels of performance at any of the ages tested. Moreover, children with ARND had faster SRTs and made fewer direction errors in the antisaccade task than children with pFAS or FAS, although all subgroups were different from controls. Our results demonstrate that eye tracking can be used as an objective measure of brain injury in FASD, revealing behavioral deficits in all three diagnostic subgroups independent of facial dysmorphology.

Introduction

Adverse outcomes occurring in offspring as a consequence of prenatal exposure to alcohol have been documented (McGee & Riley, 2006; Kodituwakku, 2007). Fetal alcohol spectrum disorder (FASD) is the umbrella term used to represent the full range of teratogenic effects attributed to gestational alcohol exposure, including fetal alcohol syndrome (FAS) (Koren *et al.*, 2003). An FAS diagnosis requires the presence of prenatal and postnatal growth restriction, craniofacial dysmorphology, and central nervous system dysfunction (Clarren & Smith, 1978; Chudley *et al.*, 2005). In the absence of one or more of these features, individuals may receive a diagnosis of partial FAS (pFAS) or alcohol-related neurodevelopmental disorder (ARND).

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Individuals with FASD may present with a range of impairments in executive function (Lezak, 1995; Funahashi, 2001), which include deficits in spatial working memory, planning, response inhibition, abstract thinking, and the ability to shift attention (Rasmussen, 2005; Kodituwakku, 2007). Impairments in executive function and social skills reported by parents and teachers demonstrate that pervasive deficits impact on behaviors across multiple settings (Schonfeld *et al.*, 2006).

Measurement of eye movement control is a powerful tool for assessing executive function (Munoz & Everling, 2004). An extensive literature based on neurophysiological, anatomical, imaging and lesion studies has contributed to our understanding of the neural circuits controlling saccadic eye movements (Heide & Kompf, 1998; Pierrot-Deseilligny *et al.*, 2004; Leigh & Zee, 2006; Sweeney *et al.*, 2007), and paradigms have been used extensively in basic and clinical research (Munoz *et al.*, 2007; Ramat *et al.*, 2007).

In this study, subjects were required to look either towards (prosaccade) or away from (antisaccade) a peripheral target that appeared on a computer monitor. Prosaccades can be triggered automatically by visual inputs to the saccade-generating circuit from the visual and posterior parietal cortices (Munoz & Everling, 2004). Antisaccades require additional steps of processing: suppression of the automatic prosaccade and initiation of the voluntary antisaccade. Successful antisaccade performance relies on circuitry that includes higher brain centers such as the frontal cortex and basal ganglia (Munoz & Everling, 2004). Deficits in parietal and frontal cortices and basal ganglia have been previously reported in FASD (McGee & Riley, 2006), making saccade tasks an appropriate tool for assessing executive function.

In a previous report (Green *et al.*, 2007c), we described eye movement abnormalities in a small cohort of children with FASD. However, the sample size was too small to allow determination of the effects of delayed development or diagnosis within the FASD spectrum on oculomotor control. To address these important questions, we developed a mobile laboratory that facilitated eye movement testing in different communities across Canada. We hypothesized that children with FASD display a developmental delay in eye movement control, such that younger children exhibit greater deficits than older children. Moreover, we predicted that differences in the magnitude of deficits in oculomotor control exist among the diagnostic subgroups, such that the children with FAS demonstrate the most profound deficits. Preliminary versions of these data have been presented in abstract form (Green *et al.*, 2007a,b).

Materials and methods

Participants

All experimental procedures were reviewed and approved by the Human Research Ethics Boards of Queen's University, the Children's Hospital of Eastern Ontario (Ottawa subjects), and the University of Alberta (Edmonton subjects). Children with FASD were recruited from eight different communities across Ontario and one community in Alberta. A total of 189 subjects were recruited into the study: 92 (40 males, 52 females; 11.2 ± 0.2 years of age; range, 8–15 years) were control children (non-FASD), and 97 were grouped as having FASD. Of the 97 subjects included in the FASD group, 89 (44 males, 45 females; 10.7 ± 0.2 years of age; range, 8–15 years) had a diagnosis within the FASD spectrum (FAS, pFAS, and ARND), and eight were suspected and/or exposed, but had yet to receive a definitive diagnosis. The children with FASD were previously assessed at local diagnostic clinics in accordance with the Canadian Diagnostic guidelines (Chudley *et al.*, 2005). Datasets from one control subject and one subject with FASD were lost because of equipment problems. All data included in the analysis for the FASD group were obtained from the 88 children who had received a diagnosis within the spectrum and 91 controls. For the purpose of analysis based on age, children were placed in one of three separate age bins: 8–10 years, 11–12 years, and 13–15 years.

Of the 89 children with FASD, 60 were medicated for behavioral symptoms related to their co-morbidities (Table 1). On the test day, primary care-givers were asked to withhold the stimulant medication until the testing was completed. Of the 38 children taking stimulant medications, eight were tested on medication. For the remaining 30 children, the last daily dose of stimulant medication was administered a minimum of 12 h prior to testing.

All control subjects had no known neurological, psychiatric or visual disorders, other than requiring corrective lenses. Primary care-givers were informed of the nature of the study, and provided written consent on behalf of the participants. All subjects completed one 1-h eye movement session. Each subject received $10 and a small gift for participating in the study.

TABLE 1. Demographic data for subjects

Category	Control (n = 92)	FASD (n = 89)
Age ± SD (years)	11.2 ± 0.2	10.7 ± 0.2
Male : female	40 : 52	44 : 45
Parent/care-giver level of education ± SD (years)	16.5 ± 0.3	14.4 ± 0.3*
Medication, n (%)		
Stimulant	0 (0)	38 (43)
Antipsychotic	0 (0)	29 (33)
Antidepressant	0 (0)	10 (11)
Anticonvulsant	0 (0)	3 (3)
Other†	12 (13)	20 (22)
Co-morbidity (10% of subjects with FASD, n (%)		
Sleeping disorders	10 (11)	55 (62)
ADHD/ADD	0 (0)	53 (60)
Oppositional defiant disorder	0 (0)	19 (21)
Anxiety	0 (0)	15 (17)
Asthma	12 (13)	10 (11)
Depression	1 (1)	10 (11)
Ratio of adults/children (home) ± SD	0.88 ± 0.04	0.76 ± 0.06*
Living with biological parents, n (%)	87 (95)	15 (17)
Parent or care-giver employed, n (%)	79 (86)	65 (73)

ADD, attention deficit disorder; AHDH, attention deficit hyperactivity disorder; FASD, fetal alcohol spectrum disorder; SD, standard deviation. *$P < 0.05$.
†Antihistamine, anti-asthma, oral contraceptives, melanin.

Saccade task

All participants performed a blocked design saccade task (Fig. 1), consisting of two blocks of prosaccade and two blocks of antisaccade trials, each consisting of 80 trials (320 trials in total). Subjects received

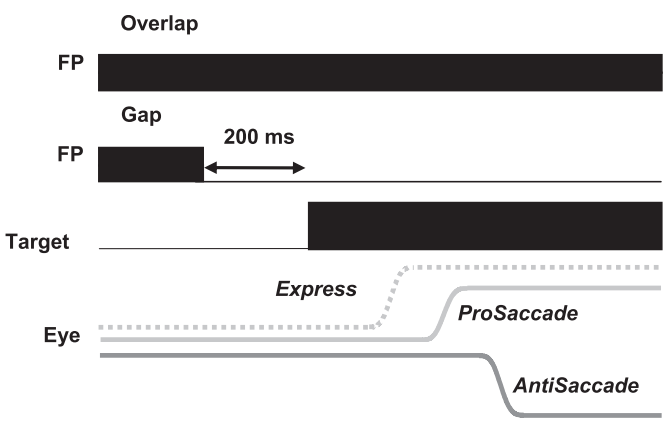

FIG. 1. In the prosaccade task, the subject was instructed to look from the central fixation point (FP) towards the eccentric target. In the antisaccade task, the subject was instructed to look away from the eccentric target to the opposite side. In both tasks, the state of fixation was manipulated such that, in the overlap condition, the FP remained illuminated while the target appeared, and in the gap condition, the FP was extinguished for a period of 200 ms before the target appeared. In both conditions, the saccadic reaction time was measured from the time of target appearance to the initiation of the first saccade.

breaks when needed, and refreshments were provided upon completion of the task. Participants were seated comfortably in a darkened room, facing the center of a laptop screen located 46 cm away. Task presentation on the laptop screen was produced using E-PRIME software (Psycholoy Software Tools, Inc., Pittsburgh, PA, USA). A red spot with a luminosity of ∼12.5 cd/m^2, and $x = 0.57$ and $y = 0.32$ coordinates in CIE space (relative to the background lumination of ∼1.0 cd/m^2, and $x = 0.34$ and $y = 0.34$ coordinates in CIE space) was positioned at the center of the screen, and served as the initial fixation point (FP). Red target spots were positioned on the screen at 15° to the right or left of the central FP. The screen was diffusely illuminated between trials, to avoid dark adaptation. Each trial began with a 250-ms period of darkness. The FP appeared for 1000 ms, and then one of two conditions occurred (Fig. 1). In the gap condition, the FP was extinguished and, after a period of 200 ms, the target appeared in the right or left visual field. In the overlap condition, the FP remained illuminated while the target appeared.

In the block of prosaccade trials, participants were instructed to look towards the target as soon as it appeared. In the block of antisaccade trials, participants were instructed to look away from the target to the opposite side. After the target had been illuminated for 1000 ms, all visual stimuli disappeared. The background illumination then reappeared, indicating the end of that trial. Target location (right or left) and fixation condition (gap or overlap) were pseudo-randomly interleaved throughout each block of trials. Subjects were asked to repeat and demonstrate the instructions to the experimenter, to ensure that they understood the task before the onset of data collection.

Recording and analysis of eye movement

The video-based infrared eye tracker (ISCAN Inc., Burlington, MA, USA) was adapted for use as a mobile laboratory, and transported to each test center. Eye position was measured using a head-mounted camera that was connected to a data acquisition computer. The video-based infrared eye tracker tracked the pupil movement, and measures of eye position and pupil size were extracted at a sampling rate of 240 Hz. Only the left eye position was digitized. Saccades were detected off-line at three standard deviations above the background, and must have lasted for longer than five sample points (MATLAB, custom software, The Mathworks, Inc., Natick, MA, USA).

Saccadic reaction time (SRT) was defined as the time from target appearance to initiation of the first saccade that exceeded 30°/s. Saccades were scored as correct if the first movement after target appearance was > 5° in amplitude and in the correct direction (i.e. towards the target for prosaccades, and away from the target for antisaccades). Saccades were scored as incorrect if the first saccade after the appearance of the target was in the wrong direction relative to the instruction (i.e. away from the target in the prosaccade, and towards the target in the antisaccade). All saccade marks and direction errors were verified off-line. The mean SRT in the prosaccade and antisaccade task was computed from all correct trials with reaction latencies between 90 and 1000 ms, to eliminate short-latency anticipatory saccades (Munoz *et al.*, 1998). In addition, we measured express saccades (latency: 90–140 ms), which are the shortest-latency visually triggered saccades (Fischer *et al.*, 1993; Dorris *et al.*, 1997); this express epoch was confirmed for the mobile laboratory. There was some variability in the experimental conditions across multiple test sites. Most notable was the amount of ambient light in which the test sessions were conducted, and we attempted to control for this variability by covering external light sources (i.e. windows) with curtains or sheets. However, target luminosity changed very little regardless of these differences in background ambient light. We also maintained similar set-up protocols to ensure that equipment and experimenter/subject space was consistent for each test site.

The following parameters were computed for each condition (gap, overlap): the mean SRT for correct trials, the coefficient of variation (CV) of SRT for correct trials [(CV = standard deviation/mean) × 100], the percentage of express saccades, and the percentage of direction errors.

Inclusion/exclusion criteria

In order to determine inclusion and exclusion criteria, SRT histograms were prepared for each subject for each experimental task (prosaccade, antisaccade) and condition (gap, overlap). On the basis of these figures, subjects were placed in bins according to their performance. For example, selection A included all subjects who could perform saccades under each task and condition, and selection E included those subjects who could only perform prosaccades. This approach provided a way of excluding subjects who could not perform certain tasks or situations where only a minimum number of trials were completed under a given condition. Univariate data analyses were conducted for each outcome measure for each task (prosaccade and antisaccade) in each condition (overlap and gap), including only the datasets from those subjects who were successful in performing the given task in the specified condition. Subsequently, analysis including both tasks and both conditions demonstrated that the statistical comparisons obtained from the complete dataset were not different from the individual univariate analyses. Therefore, statistically significant outcome measures from the complete dataset constituted a true representation of the study population.

Data analysis

The two experimental tasks (prosaccade and antisaccade) contained one within-subject factor [fixation state (gap vs. overlap)] and three between-group factors [clinical group (FASD vs. control), age (bins: 8–10 years, 11–12 years, and 13–15 years), and sex]. As attention deficit hyperactivity disorder (ADHD) results in deficits in performance of eye movement tasks (Munoz *et al.*, 2003), and ADHD was a frequently reported co-morbidity in the FASD group in this study, we included co-morbid ADHD as a covariable in the data analysis. Moreover, the impacts of medication use and parent/care-giver level of education on the performance of eye movement tasks were also tested for in the analysis. Thus, the data were first tested using multiple analysis of covariance (MANCOVA, SPSS v. 16, SPSS, Inc., Chicago, IL, USA), to examine how the dependent measures (SRT, CV, express saccades, and direction errors) were affected by the fixed factor of clinical group (control vs. FASD), and by the covariables of age, sex, co-morbid ADHD, medication use, and parental level of education. Subsequently, all dependent measures (SRT, CV, express saccades, and direction errors) were analysed using ANOVA with α set at 0.05. Difference scores (i.e. anti-effect and gap-effect) were analysed with two-tailed, unpaired Student's *t*-tests corrected with Welch's approximation when the assumption for homogeneity of variance was not met. The effect of diagnosis (ARND, pFAS, and FAS) was also determined by matching each subject in the FASD group (as closely as possible) to a control subject by age and sex. FASD and control subjects, once subdivided, were analysed by univariate analyses to test for differences between the diagnostic groups, and a Newman–Keuls *post hoc* test for multiple comparisons was conducted to contrast the pairs. Effect sizes were calculated from the means and standard deviations obtained for the major outcome measures (Cohen, 1988).

We focus on descriptions of the relevant statistical parameters for comparisons and interactions that occurred between the control and FASD groups.

Results

Consistent with previous studies (Munoz *et al.*, 1998; Dafoe *et al.*, 2007), the MANCOVA revealed significant main effects of task [prosaccade vs. antisaccade, $F_{4,169} = 40.8$, $P < 0.01$, effect size (η^2) = 0.49, power = 1] and fixation condition (gap vs. overlap, $F_{4,169} = 11.0$, $P < 0.01$, $\eta^2 = 0.21$, power = 1). There were significant interactions between task and clinical group ($F_{4,169} = 7.8$, $P < 0.01$, $\eta^2 = 0.16$, power = 1), task and age ($F_{4,169} = 16.2$, $P < 0.01$, $\eta^2 = 0.28$, power = 1), task and fixation state ($F_{4,169} = 5.0$, $P < 0.01$, $\eta^2 = 0.11$, power = 0.96), and fixation state and age ($F_{4,169} = 4.4$, $P < 0.01$, $\eta^2 = 0.21$, power = 1). The MANCOVA analysis did not reveal any effect of co-morbid ADHD, medication use or parental level of education on any of the dependent measures. We further tested the potential influence of the covariables by performing a multivariate stepwise regression analysis, which revealed a small effect of co-morbid ADHD (< 5% of the variance) for SRT, but not for any other dependent measure. As there was no significant interaction between group and fixation state, the data for the overlap and gap conditions were combined for analyses of the effect of the diagnostic subgroups.

Saccadic reaction time

Figure 2 depicts the cumulative distribution of SRT for correct responses (positive values) and direction errors (negative values) in all experimental conditions for control children and those diagnosed with ARND, pFAS, or FAS. Children with FASD had longer SRTs than controls ($F_{1,165} = 18.6$, $P < 0.001$). The anti-effect (anti-SRT – pro-SRT) provides a measure of the difference in reaction times for antisaccades and prosaccades, thus illustrating differences in the voluntary and automatic mechanisms. The anti-effect for children with FASD was not significantly different from that of control children in the overlap or gap conditions ($P > 0.05$). The mean anti-effects were 100 ± 7 ms and 119 ± 7 ms for children with FASD, and 94 ± 5 ms and 109 ± 4 ms for controls, in the overlap and gap conditions, respectively.

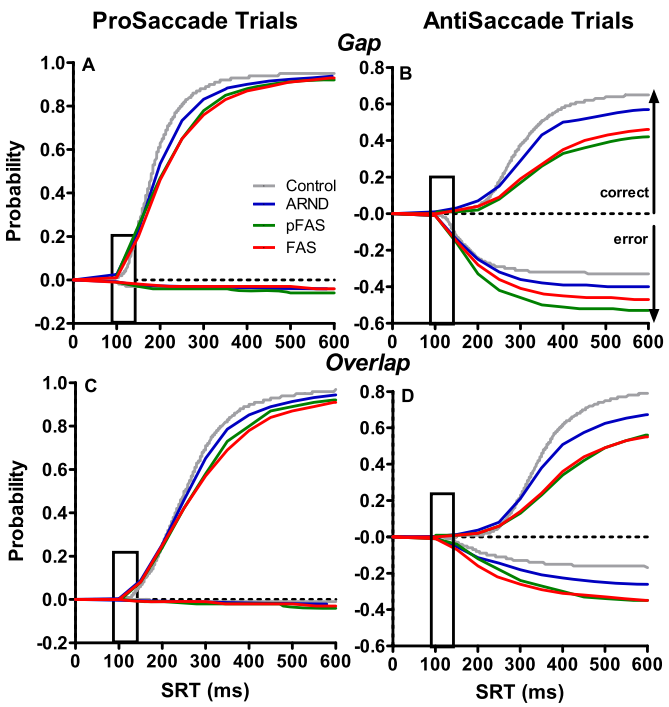

FIG. 2. Cumulative distribution of saccadic reaction times (SRTs) for correct responses (positive values on the ordinate) and direction errors (negative values on the ordinate) for prosaccade (A and C) and antisaccade (B and D) trials in the gap (A and B) and overlap (C and D) conditions. ARND, alcohol-related neurodevelopmental disorder data; pFAS, partial fetal alcohol syndrome data; FAS, fetal alcohol syndrome data. The open box highlights the express saccade epoch (90–140 ms).

The gap-effect (overlap SRT – gap SRT) provides a measure of the difference between fixation conditions, and serves to illustrate whether there are deficits in the processes of disengagement from fixation. The mean gap-effects for prosaccades were 71 ± 4 ms for children with FASD and 75 ± 3 ms for control subjects, and there was no significant difference between groups ($P > 0.05$). Similarly, the gap-effect for antisaccades was also not significantly different between the two groups ($P > 0.05$), and the means were 51 ± 7 ms and 60 ± 2 ms for FASD and control subjects, respectively.

After pairing of each child within the diagnostic subgroup with the appropriate control, unpaired *t*-tests were conducted. In the prosaccade task, complete datasets were obtained from 42 children with ARND, 18 with pFAS, and 25 with FAS; in the antisaccade task, there were 41 children with ARND, 18 with pFAS, and 24 with FAS. In comparison to their matched controls, children with ARND had longer prosaccade SRTs ($t_{80} = 2.6$, $P < 0.05$), but were not different for antisaccade SRTs ($P > 0.05$), although the scores approached significance ($P = 0.06$). Children with pFAS were not significantly different from their matched controls with respect to prosaccade SRTs ($P > 0.05$), but did have longer antisaccade SRTs ($t_{36} = 3.1$, $P < 0.01$). As compared to their matched controls, children with FAS demonstrated longer prosaccade SRTs ($t_{61} = 3.1$, $P < 0.01$) and antisaccade SRTs ($t_{45} = 3.6$, $P < 0.01$).

We were also interested in determining whether there were significant differences between children with ARND, pFAS and FAS across the different outcome measures. There were no significant differences among the diagnostic subgroups for prosaccade SRTs (Fig. 3A). However, there was a significant difference for antisaccade SRT ($F_{2,79} = 5.7$, $P < 0.01$), such that children with pFAS and FAS had longer SRTs than children with ARND ($P < 0.05$) (Fig. 3D).

CV SRT

The CV expresses the intra-subject variability in SRT. Children with FASD demonstrated greater variability than controls ($F_{1,165} = 32.0$, $P < 0.001$). This difference in SRT variability among children in the FASD group is probably due to increased heterogeneity in task performance resulting from differing degrees of brain injury and subsequent dysfunction following prenatal alcohol exposure.

In comparison to their matched control groups, children with ARND or pFAS were not different for prosaccade CV ($P > 0.05$; Fig. 3B). In contrast, children with ARND ($t_{79} = 3.7$, $P < 0.01$) and pFAS ($t_{36} = 3.3$, $P < 0.01$) were different from their matched control groups for antisaccade CV (Fig. 3E). As compared to their matched controls, children with FAS demonstrated greater prosaccade CV ($t_{46} = 3.2$, $P < 0.01$) and antisaccade CV ($t_{45} = 3.7$, $P < 0.01$) (Fig. 3B and E).

Among the diagnostic subgroups, there were no significant differences in CV for prosaccades or antisaccades (Fig. 3B and E) ($P > 0.05$).

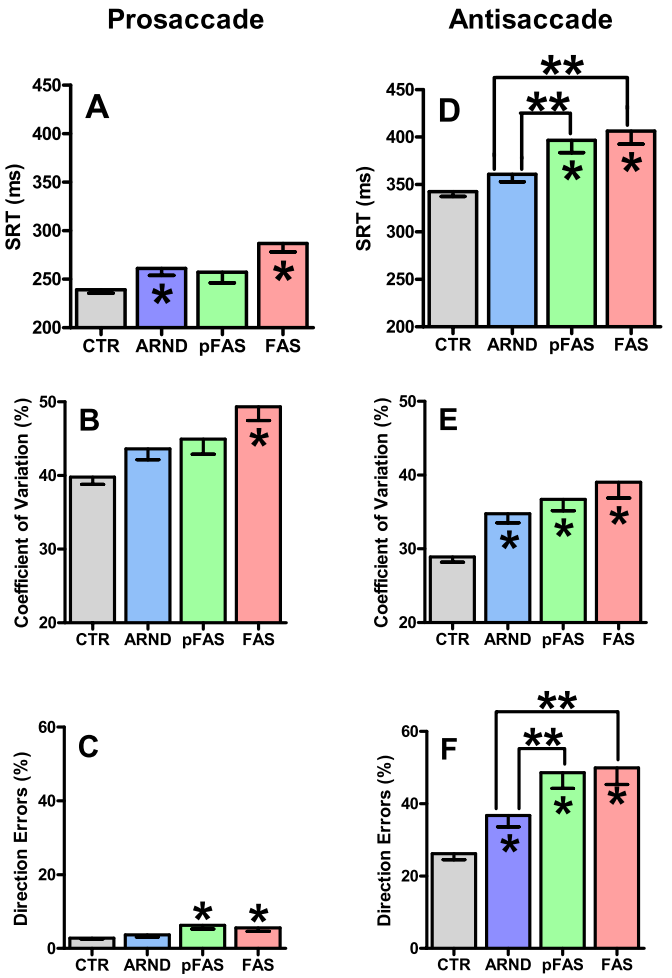

FIG. 3. Quantification of parameters for the prosaccade (A–C) and antisaccade (D–F) tasks. (A and D) Mean saccadic reaction times (SRTs) for correct responses. (B and E) Coefficient of variation in SRT [(standard deviation of SRT/mean SRT) × 100%]. (C and E) Percentage of direction errors. CTR, control data (subgroups combined); ARND, alcohol-related neurodevelopmental disorder data; pFAS, partial fetal alcohol syndrome data; FAS, fetal alcohol syndrome data. *$P = 0.05$ as compared with matched-control subjects; **$P < 0.05$ for ARND difference from pFAS and FAS.

Express saccades

In contrast to our previous findings (Green *et al.*, 2007c), where children with FASD generated significantly fewer express saccades, there was no effect of either clinical group (control vs. FASD) or diagnostic subgroup (ARND, pFAS, FAS) on the proportion of express saccades ($P > 0.05$) (data not shown).

Direction errors

Children with FASD made more direction errors than controls ($F_{1,165} = 30.5$, $P < 0.001$). In comparison to their matched control group, children with ARND were not different for direction errors in the prosaccade task, although the difference approached significance ($P = 0.055$). As compared with their respective control groups, children with pFAS ($t_{36} = 3.7$, $P < 0.01$) or FAS ($t_{46} = 2.0$, $P < 0.05$) made more direction errors in the prosaccade task (Fig. 3C). In the antisaccade task, children with ARND ($t_{79} = 3.8$, $P < 0.01$), pFAS ($t_{36} = 2.7$, $P < 0.05$) or FAS ($t_{45} = 4.0$, $P < 0.01$) all made more direction errors than their matched control groups (Fig. 3F).

Among the diagnostic subgroups, there were no significant differences in the percentage of direction errors for prosaccades (Fig. 3C) ($P > 0.05$). In contrast, there was a significant difference between the diagnostic subgroups for errors in the antisaccade task ($F_{2,79} = 3.9$, $P < 0.05$), such that children with ARND made fewer direction errors than the children with pFAS or FAS (Fig. 3F).

Age

To examine the effect of age, children in the two experimental groups (controls and FASD) were distributed into different age bins: 8–10 years, 11–12 years, and 13–15 years. The ANOVA revealed a significant effect of age for SRT ($F_{2,165} = 11.2$, $P < 0.001$), CV ($F_{2,165} = 9.6$, $P < 0.001$), and direction errors ($F_{2,165} = 13.5$, $P < 0.001$), but not for express saccades ($F_{2,165} = 0.4$, $P = 0.6$). Consistent with previous studies (Munoz *et al.*, 1998), performance in these tasks improved across the range of ages tested for children with FASD and controls, as observed for antisaccade SRT and percentage of direction errors in the gap and overlap conditions (Fig. 4). The same observations were made for antisaccade CV, as well as prosaccade SRT, CV, and percentage of direction errors (not shown). However, there was no interaction between age and group, which suggests that deficits in oculomotor control in children with FASD cannot be explained by developmental delay alone, as they failed to achieve age-matched control levels of performance.

Effect size

The effect size was calculated for the dependent measures (SRT, CV, express saccades, and direction errors) for both prosaccade and

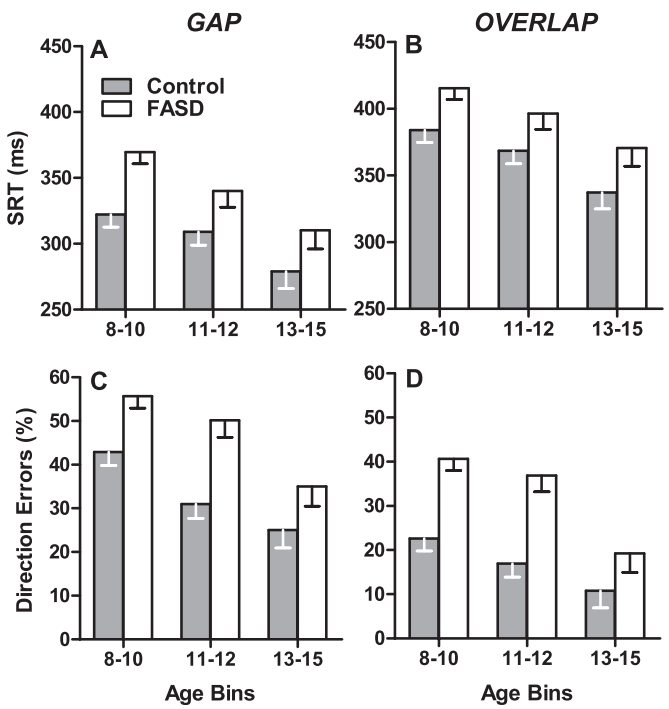

FIG. 4. Mean saccadic reaction times (SRTs) (A and B) and direction errors (C and D) vs. age for the antisaccade task in the gap (A and C) and overlap (B and D) conditions. Shaded bars, control data; open bars, fetal alcohol spectrum disorders (FASD) data.

TABLE 2. Effect size for eye movement outcome measures

Task	Cohen's *d*	Effect-size (*r*)
Prosaccade SRT	−0.64	−0.31
Prosaccade coefficient of variation	−0.59	−0.28
Prosaccade express saccades	0.07	0.04
Prosaccade direction errors	−0.60	−0.29
Antisaccade SRT	−0.69	−0.33
Antisaccade coefficient of variation	−0.99	−0.44
Antisaccade direction errors	−0.92	−0.42

SRT, saccadic reaction time.

antisaccade tasks (Table 2). With the exception of express saccades, these outcome measures demonstrated moderate to large effect sizes (0.5–0.99), indicating a significant degree of non-overlap in the performance of the two groups.

Discussion

In this study, subjects were required to look either towards (prosaccade) or away from (antisaccade) a peripheral target that appeared on a computer monitor. The former probes the ability of subjects to generate automatic visually triggered saccades, and the latter tests the ability to suppress the automatic saccade and generate a voluntary response in the opposite direction. Children with FASD exhibited increased saccadic reaction times, increased intra-subject variability, and increased direction errors. We also demonstrated that the greatest magnitude of difference in performance across the diagnostic subgroups occurred for antisaccade tasks, which reflects deficits in executive function (Munoz & Everling, 2004). Moreover, children with FASD never achieved a level of performance equivalent to that of the age-matched control group, which suggests that deficits in eye movement control may persist into adulthood (Chudley *et al.*, 2007). We discuss these findings as they relate to the current understanding of oculomotor control and diagnostic subgroups of FASD.

Oculomotor circuitry

The oculomotor system has been well characterized (Heide & Kompf, 1998; Munoz & Everling, 2004; Pierrot-Deseilligny *et al.*, 2004; Sweeney *et al.*, 2007). The main cortical areas involved in saccade generation are the parietal eye field located in the posterior parietal cortex (PPC), the frontal eye fields (FEFs), the dorsolateral prefrontal cortex (dlPFC), and the supplementary eye fields (SEFs) in the frontal lobe (Munoz & Everling, 2004), all of which project directly to the intermediate layers of the superior colliculus (SCi) to control saccade production. Oculomotor areas of the frontal cortex also send projections to the SCi via the direct, indirect and hyperdirect pathways through the basal ganglia (Hikosaka *et al.*, 2000; Nambu *et al.*, 2002; Munoz & Everling, 2004; Munoz *et al.*, 2007). The basal ganglia are generally associated with cognitive and motor function, and play a key role in oculomotor control (Hikosaka *et al.*, 2000). The caudate nucleus is related to oculomotor behaviors that are necessary for predicting environmental changes (Hikosaka *et al.*, 1989; Cameron *et al.*, 2007). Decreased activity in this component of the basal ganglia may impede performance even in simple oculomotor tasks such as the prosaccade and antisaccade tasks.

Parietal eye field lesions produce increased prosaccade latencies, with little effect on volitional saccades in monkeys (Lynch & McLaren, 1989); unilateral lesions to the PPC increase prosaccade latency in both the gap and overlap conditions in humans (Pierrot-Deseilligny *et al.*, 1987, 1991b). Patients with lesions to the FEF demonstrate profound difficulties in initiating antisaccades, leading to elevated SRTs (Rivaud *et al.*, 1994; Gaymard *et al.*, 1999), suggesting its critical role in the initiation of intentional voluntary saccades. Lesions to the dlPFC lead to an increase in direction errors (i.e. automatic prosaccades) in the antisaccade paradigm, whereas prosaccades are relatively unaffected (Guitton *et al.*, 1985; Pierrot-Deseilligny *et al.*, 1991a). The SEF is important for saccade sequences by combining or coordinating voluntary saccades, and may be important for generation of successful antisaccades (Schlag-Rey *et al.*, 1997; Gaymard *et al.*, 1998).

The results from our study demonstrate two areas of deficient oculomotor control in children with FASD: (i) saccade initiation leading to increased SRTs; and (ii) saccade suppression resulting in increased direction errors in the antisaccade task. These deficits are consistent with damage to basal ganglia and parietal and frontal cortices. Structural magnetic resonance imaging (MRI) studies have demonstrated a number of abnormalities following prenatal alcohol exposure: (i) a disproportionate reduction in the parietal lobe (Archibald *et al.*, 2001); (ii) a relative increase in gray matter and decrease in white matter in the perisylvian cortex of the parietal lobes (Sowell *et al.*, 2001); (iii) reduced brain growth in the frontal lobes, including the orbitofrontal cortex (Riley *et al.*, 2004); and (iv) decreased basal ganglia volumes, with specific reductions in the caudate nucleus (Mattson *et al.*, 1996). Decreased caudate activity has also been shown using the blood oxygenation level-dependent signal from functional MRI studies in subjects with FASD following tasks that require inhibitory control (Fryer *et al.*, 2007). Taken together, these findings indicate that prenatal alcohol exposure has prolonged effects on brain development long after the *in utero* insult. These results are consistent with the known deficits in executive function associated with FASD (Rasmussen, 2005), and implicate the basal ganglia and parietal and frontal cortices as areas of particular sensitivity to prenatal ethanol exposure.

To summarize, PPC damage probably contributes to the increased SRTs observed for prosaccades in children with FASD, whereas damage to frontal structures (FEF, SEF, and dlPFC) and basal ganglia lead to increased SRTs for antisaccades and reduced ability to suppress automatic saccades. Downstream structures such as the SCi are probably affected only indirectly via aberrant projections from the frontal or parietal cortices or basal ganglia. On the basis of the normal prosaccade metrics in FASD (Green *et al.*, 2007c) and the normal gap-effect (this study), it appears that the SCi and brainstem saccade-generating circuits remain structurally intact (Leigh & Zee, 2006), and the functional abnormalities are due to atypical connections arising from upstream structures. We attribute the increased direction errors observed in the prosaccade task to difficulties in focused attention in children with FASD. Future functional imaging studies using the same oculomotor tasks will confirm or refute the extent of involvement of these structures, and provide more definitive answers.

In contrast to our previous report (Green *et al.*, 2007c), children with FASD did not execute fewer express saccades than controls. This observation was not attributed to sudden performance improvement by the children with FASD; rather, it was due to the control subjects, who generated fewer express saccades under the experimental conditions used in the mobile laboratory. In our previous study, complete darkness was achieved during experimental testing; however, during target presentation, the same conditions were not possible using the mobile laboratory, and the presence of ambient lumination probably underlies this result. These observations warrant further investigation.

Developmental delay and FASD subgroups

This large-scale study allowed us to address questions related to the effects of age and diagnostic subgroup on oculomotor behavior in children with FASD. There was no age by group interaction in performance of the oculomotor tasks. Although there was an improvement with age, subjects with FASD failed to achieve age-matched control levels of task performance at any of the ages tested. This suggests that the deficits in oculomotor control cannot be explained by developmental delay alone; they are probably attributable to brain injury that persists well into adulthood (Chudley *et al.*, 2007), involving dysfunction of the frontal–striatal circuitry.

We postulated that eye movement testing would reveal differences in the magnitude of deficits among the diagnostic subgroups (i.e. FAS, pFAS, and ARND). For instance, we expected that children with FAS, who are considered to be at the more severe end of the spectrum, would exhibit the greatest magnitude of deficits in eye movement control. This postulate was supported by the data obtained for the antisaccade task, which revealed that children with ARND had shorter SRTs, and made fewer direction errors, than children with pFAS or FAS. On a number of neuropsychological tests that probe aspects of executive function, published studies of children prenatally exposed to alcohol have reported no performance differences between dysmorphic and non-dysmorphic children (Mattson *et al.*, 1999; Schonfeld *et al.*, 2006). Alcohol-exposed individuals with and without facial features exhibited statistically significant increases in cortical thickness, demonstrating that the facial phenotype was not a prerequisite for brain dysmorphology (Sowell *et al.*, 2007). In a functional MRI study, response inhibition in children and adults with heavy prenatal alcohol exposure showed no significant differences in the regions of interest between individuals with and without an FAS diagnosis, although both groups were significantly different from control subjects (Fryer *et al.*, 2007). Notably, children with ARND are most difficult to diagnose in a clinical situation, as they lack the facial dysmorphology (Chudley *et al.*, 2005). Although we found differences between the diagnostic subgroups in the antisaccade task, all subgroups were different from their age-matched controls, even the children with ARND (Fig. 3). Thus, measuring deficits in eye movement control may have significant potential for screening individuals at risk for FASD.

Study limitations

The majority of children in the FASD group (83%) were living in foster or adoptive homes, and in the majority of cases (74%) the primary care-giver was employed at the time of testing. Information on medical and family histories, including drug and alcohol abuse by first-generation relatives, was collected for each participant in the study. However, for a large proportion of the children in the FASD group (those in foster or adoptive homes), information on maternal and paternal drug and alcohol abuse was not available, which prevents us from examining the impact of family history on the performance of eye movement tasks in our study group. A positive family history of alcohol abuse has been found to influence some, but not all, parameters of eye movement tasks (Blekher *et al.*, 2002) and to contribute to an increase in the number of impulsive errors in executive function tasks (Saunders *et al.*, 2008). Thus, the inability to examine this potential confound is a limitation of the current study. Co-morbidities, in particular ADHD, occur with high frequency in children with FASD (Table 1). However, in the current study we found that co-morbid ADHD could not account for the deficits in performance of eye movement tasks found for the FASD group. Interestingly, there was a small but statistically significant contribution of co-morbid ADHD on SRT, which suggests that co-morbid disorders may contribute to differing patterns of behavioral deficits in eye movement control in children with FASD. We are currently conducting a separate study to more thoroughly investigate the potential contribution of co-morbid disorders such as ADHD to the deficits in eye movement behaviors observed in children with FASD.

Conclusion

Saccadic eye movement tasks show promise for assessing the brain injury resulting from prenatal exposure to alcohol. Children between the ages of 8 and 15 years demonstrated profound deficits across many outcome measures for both prosaccade and antisaccade tasks, suggesting dysfunction in frontal and parietal cortices and the basal ganglia. Thus, eye movement experiments, and particularly the antisaccade task, provide objective measures of executive dysfunction in children with FASD and may provide a more sensitive measure of overall cognitive function. This is an important point, as it has been shown that performance across tasks of executive function were lower in FASD than would be otherwise predicted by IQ alone, supporting the need for novel tools that can provide sensitive and specific assessments of brain injury (Niccols, 2007). With the advent of eye tracker systems equipped for use in MRI, it will be possible to identify the specific cortical and subcortical regions underlying these deficits.

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Abbreviations

ADHD, attention deficit hyperactivity disorder; ARND, alcohol-related neurodevelopmental disorder; CV, coefficient of variation; dlPFC, dorsolateral prefrontal cortex; FAS, fetal alcohol syndrome; FASD, fetal alcohol spectrum disorder; FEF, frontal eye field; FP, fixation point; MRI, magnetic resonance imaging; pFAS, partial fetal alcohol syndrome; PPC, posterior parietal cortex; SCi, intermediate layers of the superior colliculus; SEF, supplementary eye field; SRT, saccadic reaction time.

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